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PERKIN-ELMER

993-9500

LC-75 SPECTROPHOTOMETRIC DETECTOR FOR LIQUID CHROMATOGRAPHY

February 1979

PERKIN-ELMER NORWALK, CONNECTICUT, U.S.A.

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CAUTION

All portions of the instrument which come in direct contact with the solvent are made of 316 stainless steel (or equivalent), PTFE, glass, gold/nickel alloy, or sapphire. Some of these materials are extremely sensitive to acidic chlorides.

> **Important:** Do not permit acidic chlorides (those with pH less than or equal to 7) to come in contact with any of the internal portions of the instrument. Instrument corrosion or failure resulting from the use of acidic chlorides is NOT covered by the warranty.

For example, some of the internal portions of the instrument are extremely sensitive to hydrochloric acid. Several publications have described the use of "TRIZMA" buffers in ion exchange work. These are available in several forms. The acid form Tris (hydroxymethyl) aminomethane HCl (TRIS-HCl), contains hydrochloric acid and MUST NOT BE USED. The pH conditions required to duplicate most published separations can be met with the phorphoric version, Mono-Tris (hydroxymethyl) aminomethane phosphate (TRIS-H_3PO_4).

In addition, some users of LC systems have observed that chloroform and carbon tetrachloride, although not ionized themselves, slowly decompose to liberate HCl. HCl, as noted above, attacks stainless steel. Do not leave these solvents in the system for a prolonged period if the system is not being used. The first symptom of the decomposition of chloroform or carbon tetrachloride is a blue-green system effluent. Should this occur, thoroughly flush the entire system with methanol and then with water. Then call your Perkin-Elmer Service representative to receive instructions on re-passivating your system.

1 INTRODUCTION

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INTRODUCTION

1A. SCOPE OF THE MANUAL

This instruction manual has been provided to acquaint the user with the LC-75 Spectrophotometric Detector. The manual assumes that the user has developed a working knowledge of liquid chromatography.

A working knowledge of liquid chromatography includes the following:

a) Knowing the function of each major component in a liquid chromatography system;

b) The ability to choose a column based on the type of analysis to be done;

c) Understanding the principles of solvent selection and sample preparation;

d) Understanding the term "UV cutoff";

Should the user require an introduction to these concepts, the Appendices at the end of these instructions provide fundamental information which may prove useful. Further information, outside of the scope of these instructions, may also be found in Introduction to Modern Liquid Chromatography by Snyder and Kirkland, published by Wiley-Interscience (New York, 1974).

The first purpose of the manual is to provide relevant operating instructions for the LC-75. For the user's convenience, a durable copy of the operating summary is included at the back of Section 7. Installation, routine maintenance, and service procedures are included in these instructions in addition to other useful data.

Fig. 1-1 - LC-75 Detector

1B. DESCRIPTION

The Perkin-Elmer Model LC-75 Spectrophotometric Detector is a single beam, variable wavelength detector, designed for use in a liquid chromatography system. The detector is a self-contained unit as shown in Figure 1-1 and houses a variable wavelength spectrophotometer, a high-pressure flow-through sample cell, and the electronics required to control the unit.

The detector permits absorbance detection in the ultraviolet-visible range (190 nm to 600 nm). The spectrophotometer is insensitive to flow noise and to changes in the refractive index of solvents.

The microcell assembly has a cell volume of 8 microliters and can withstand pressures of 14 Mega Pascals (2000 psig). This capability is required when the flow from the cell is stopped, or when back pressure is applied deliberately to prevent the formation of gas bubbles during gradient elution. (See Section 2B,2.)

The Model LC-75 can be used with any laboratory recorder requiring a 10 mV input voltage. A recorder with an input range of 0 to 10 mV (full scale) is required. Perkin-Elmer Recorder Models 023 and 056 are recommended.

2 INSTALLATION

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2 INSTALLATION

2A. PREPARATION

2A,1. General Requirements

Prepare a sturdy bench location in a clean environment with relatively stable temperature and humidity. The detector will operate within the specifications when the ambient temperature ranges from 19 to 30 degrees Celsius. Ambient relative humidity is considered to be from 20 to 80% with no condensation.

2A,2. Electrical Requirements

The electrical supply system must be within ±10% voltage tolerance of the nominal voltage for the particular detector in use. A printed circuit board below the line fuse on the LC-75 left side permits the instrument to be used at one of four nominal line voltages. See Section 2C,2. A good earth ground is also required, common to all instrument modules and user-supplied attachments. The grounding must be accomplished by a third wire in the electrical supply system. The detector should not be attached to circuits associated with equipment of a heavy-duty nature or large or frequent loads as this may result in excessive noise which may interfere with the detector signal.

2A,3. Gas Requirements

The detector optical paths are enclosed and purgeable with dry nitrogen to remove UV absorbing solvents. Nitrogen used for this purpose must be pure, clean, dust-free, oxygen-free and preferably boiled-off from liquid nitrogen. Pre-purified tank nitrogen is acceptable. Use 1/4-in. fluorocarbon tubing (e.g. Teflon) or equivalent for the nitrogen purge line. DO NOT USE RUBBER OR OTHER TYPES OF PLASTIC TUBING. (See Section 2B,3.)

2A,4. Parts Provided

These items, included in Spares Kit 271-0265, are shipped with the LC-75 Detector. Those items which are not used during the installation should be stored, in a clean, safe location for future use.

P-E Part No.	Description	Quantity
087-3177	Cell Window	2
087-2234	Spring Disk	6
087-1130	Cell Window Seal	6
087-3140	Teflon Tubing, 17 ga., 6 ft.	1
087-3031	1/16-in. Nut, Parker-Hannifin	1
087-3032	1/16-in. Ferrule, Parker-Hannifin	1
087-3036	1/16-in. O.D. (.015-in. I.D.) S.S. Tubing, 2 meter	1
254-0838	Restrictor Coil Assembly, 1/16-in. O.D. (.007-in. I.D.)	2
990-7225	Screwdriver	1
990-7271	Allen Wrench, 3/32 in.	1
990-3274	Coupling	2
990-3250	1/16-in. L.P. Collar, S.S.I.	4
990-3270	1/16-in. L.P. Gland Nut, S.S.I.	4
998-1622	Fuse, Int. H.V., 0.5A	1
998-1762	Fuse, 0.8A, Slo-Blo	1

P-E Part No.	Description	Quantity
998-1624	Fuse, 1.5A, Slo-Blo	1
254-0943	Recorder Cable Assembly	1
993-9500	Instruction Manual	1

While this Kit is not intended to include all the replacement parts which may be required eventually, the listing may be used as a partial list of replacements.

2B. TUBING CONNECTIONS

2B,1. General Information

Perkin-Elmer liquid chromatography systems utilize 1/8-in. tubing and fittings upstream of the pump(s) and 1/16-in. tubing and fittings after the pump(s). (SSI type fittings are used on the Model LC-75.) All of the 1/16-in. tubing is #316 stainless steel or equivalent. When you assemble any of the tubing required in your system, scribe the tubing squarely with a triangular or blade file. Then use two smooth-jaw pliers to separate the pieces of tubing. (See Figure 2-1.) Gently turn the pliers in opposing directions to separate the tubing, gently bending the tube as required. The tubing ends must be free of burrs and snags. Use Figure 2-2 as a guide when you orient the nut and its associated ferrule(s) or collar. Note that the tubing must project past the collar or ferrule. Flush new tubing to clean.

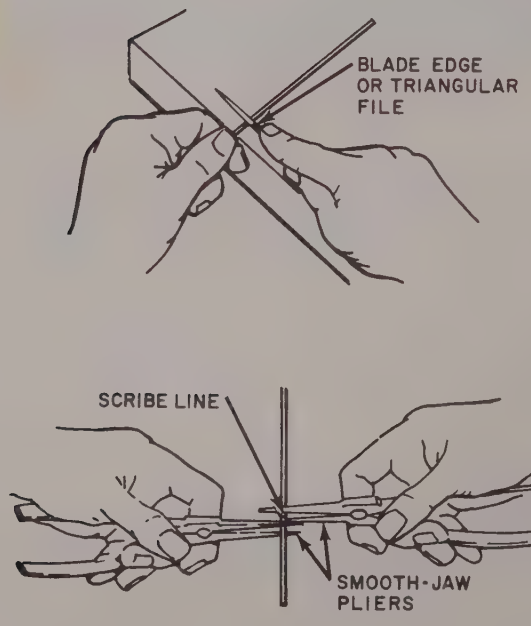


Fig. 2-1 - Cutting Stainless Steel Tubing

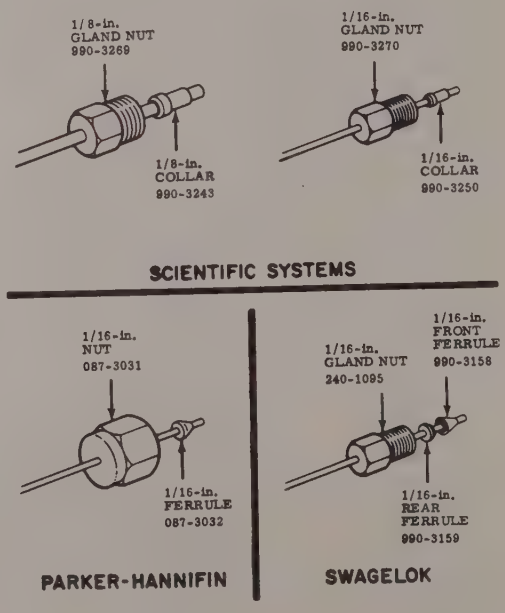


Fig. 2-2 - Orientation of Hardware

When you make the connection, insert the tubing and collar into the fitting seat. Press and hold the tubing to the seat bottom. Turn the nut into the fitting following the nut with the wrench carefully. Never over-tighten the fitting. Fingertighten the nut and then use a wrench to tighten it no more than 90 degrees past fingertight when the fitting is new (45 degrees past fingertight when the fitting is being reconnected).

Turn on the system and bring the system to its normal operating pressure after making any plumbing modifications. If leaks occur, tighten the fittings an additional 10 degrees. If the leaks persist, disassemble the affected fitting(s) and inspect the fitting(s). The threads should be in good condition and the collar or ferrule(s) should be uniformly deformed. The collar or ferrule(s) should grasp the tubing firmly. If the hardware appears to be in good condition, remake the connnection, and tighten to the required level. If the leak still persists, replace the hardware and repeat the procedure until leak-free operation is achieved.

2B,2. Detector Solvent Lines

One length of unassembled stainless steel tubing and two restrictor coil assemblies are provided in the Spares Kit shipped with the LC-75. The longer piece bears the part number 087-3036 and is 0.015-in. I.D. tubing while the shorter (1 m) coils (part number 254-0838) are 0.007-in. I.D. tubing. Either tubing may be used to connect the column outlet to the detector inlet line and the detector outlet to another detector. In either case, unions of the zero-dead-volume-type should be used to make the connection. The 0.015-in. I.D. tubing is generally used with higher flow rates; 0.007-in. I.D. tubing is used only at low flow rates. The smaller bore tubing is advantageous when working with high efficiency columns (equivalent to 10,000 theoretical plates). Although the flow cell in the LC-75 can withstand relatively high pressures, there are practical limits which depend on the type of column(s) used, and the flow rate delivered by the pump(s). Consult Table 2-1 to determine the tubing size which can be used.

TABLE 2-1
1/16-IN. TUBING I.D. USE AND SYSTEM CONDITIONS

COLUMN PACKING SIZE	FLOW RATE	
	below 2.0 ml/min	over 2.0 ml/min.
10 micrometer	0.007 in. I.D.	0.015 in. I.D.
5 micrometer	0.007 in. I.D.	0.015 in. I.D.

CAUTION: When 0.007-in. I.D. tubing has been used anywhere in the system, operate the pump(s) at low flow rates only. High flow rates and the resulting higher pressure on the cell may damage the cell. In addition, always filter the solvent.

Standard 1/16-in. fittings may be used throughout the system. Use of standard 0.015-in. I.D. couplings in cases where 0.007-in. I.D. tubing is used will introduce a minute volume area which may trap a portion of the sample. This condition should not affect the analysis due to the small area involved.

The two couplings with Perkin-Elmer part number 990-3274 have a 0.015-in. inner bore which is acceptable in 99% of the work done in liquid chromatography today. In extreme cases involving very exact analytical work which will suffer from the introduction of any dead volume in the couplings, use 0.007-in. inner bore dead-volume couplings if available when using 0.007-in. tubing into the system.

Generally, use the two-meter length of 1/16-in. tubing provided in the Spares Kit, to connect the detector inlet to the column outlet. Cut the tubing to the proper length. Make certain that the tubing is square and free of burrs. Use the Parker-Hannifin nut (087-3031) and ferrule (087-3032) at the column connection. Use the S.S.I. hardware at the detector INlet. In a similar fashion, use the remaining length at the detector OUTput. You may use this as a drain (provide a waste container) or you may connect the solvent line to another detector. Hardware to make connection to another detector is not included with the LC-75. Tighten all the connections no more than 90 degrees past fingertight.

The restrictor coil assembly (P-E part no. 254-0838) includes 0.007-in. I.D. tubing and is used to apply system back pressure to aid in reducing the formation of gas bubbles in the cell and the resulting peak broadening during gradient elution work.

The other restrictor coil assembly may be unwound and used between the column outlet and the detector inlet when a high efficiency column is in use at low flow rates only.

Important: Always filter the solvent when using the restrictor coil assembly at the detector outlet to apply system back pressure. Solvent filtering decreases the chances of a blockage in the restrictor coil producing a system back pressure in excess of the maximum 13.4 kPa (2000 psi) pressure permissible. As a further safeguard, it is advisable to use the 0.007-in. I.D. tubing between the column outlet and the detector inlet.

2B,3. Detector Purge

2B,3a. Monochromator Purge

Depending upon the type of analyses performed, it will be necessary to purge the monchromator area to remove residual UV-absorbing solvents. Only pre-purified nitrogen should be used. Alternatively an effective moisture and particulate scrubber must be installed in the line from the tank to the detector. See Figure 2-3 to locate the purge hose fitting. Use a 25 cm (9 to 10-in) length of 0.016-in. stainless steel tubing.

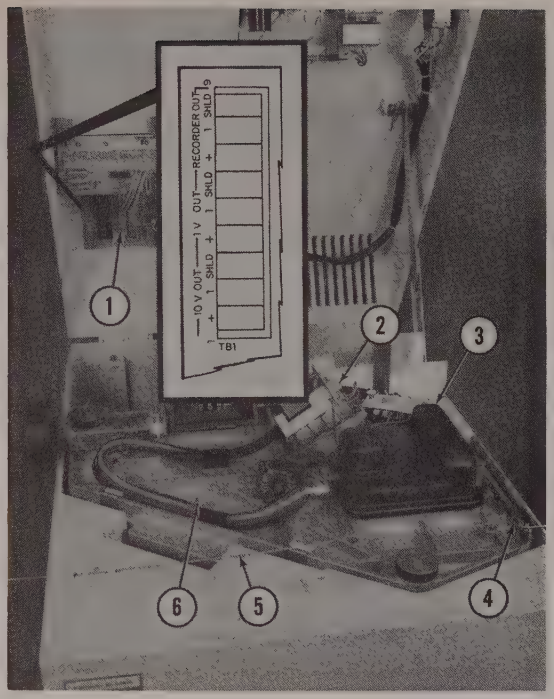
1) Carefully form a right-angle bend in the tubing so that one leg is approximately 10 cm (3 1/2-in.) and the other leg is about 15 cm (5 1/2-in.) long.

2) Insert the longer leg into the hose fitting and slide 1/4-in. Teflon tubing over the other end of the stainless steel tubing.

3) Use appropriate fittings to connect the nitrogen line to the Teflon tubing.

> **Important**: Use only stainless steel tubing and fluorocarbon (Teflon) tubing in the nitrogen line. Do not use rubber or any other type of plastic tubing.

4) Nitrogen regulated at 5 psi must be clean and dry. Preferably use boiled-off liquid nitrogen.



1 TB1
2 Source
3 Cell Area
4 Solvent INlet Line
5 Solvent OUTlet Line
6 Purge Hose Fitting

Fig. 2-3 - LC-75 (Shown with Cover Lifted)

2B,3b. Cell Purge

From time to time it will be necessary to purge the cell area. This is particularly important when the detector performance is checked as outlined in Sections 8 and 11.

1) Remove the column from the system, and install a dummy (empty) column. Use 0.015-in. I.D. tubing from the dummy column to the detector inlet.

2) Pump isopropanol through the system for five minutes. Use a flow of 6 ml/minute. Shut off the pump.

3) Disconnect the pump outlet from the detector inlet and connect the nitrogen supply to the LC-75 inlet.

> **Important**: Use only stainless steel tubing and fluorocarbon (Teflon) tubing for the nitrogen line. Do not use rubber tubing or any other plastic tubing.

4) Empty a slight nitrogen purge (never more than 40 psi) to flush the cell. Use clean and dry, preferably pre-purified, nitrogen for the purge.

2C. ELECTRICAL CONNECTIONS

2C,1. Recorder and Accessory Connections

Use the recorder cable (P-E part no. 254-0943) provided to make the appropriate recorder connections at TB501, located on the underside of the detector cover. (See Fig. 2-3.)

Use the recorder cable to make these connections to a 10 mV recorder:

TB1-7 to Recorder Input (+)
TB1-8 to Recorder Input (-)

If a 1 V or 10 V data acquisition device will be used, use a shielded two-conductor cable or use the recorder cable to make connection to:

TB1-4 to Data Input
TB1-5 to Return
TB1-6 to Shield

For example, the Perkin-Elmer Model Sigma Console connnection is given in Figure 2-4. Connect red to TB1-4, black to TB1-5 and the shield to TB1-6 if the marker cable is used. The output signal is 1V for 10.24 A_{1cm} where A_{1cm} is absorbance normalized to 1cm pathlength.

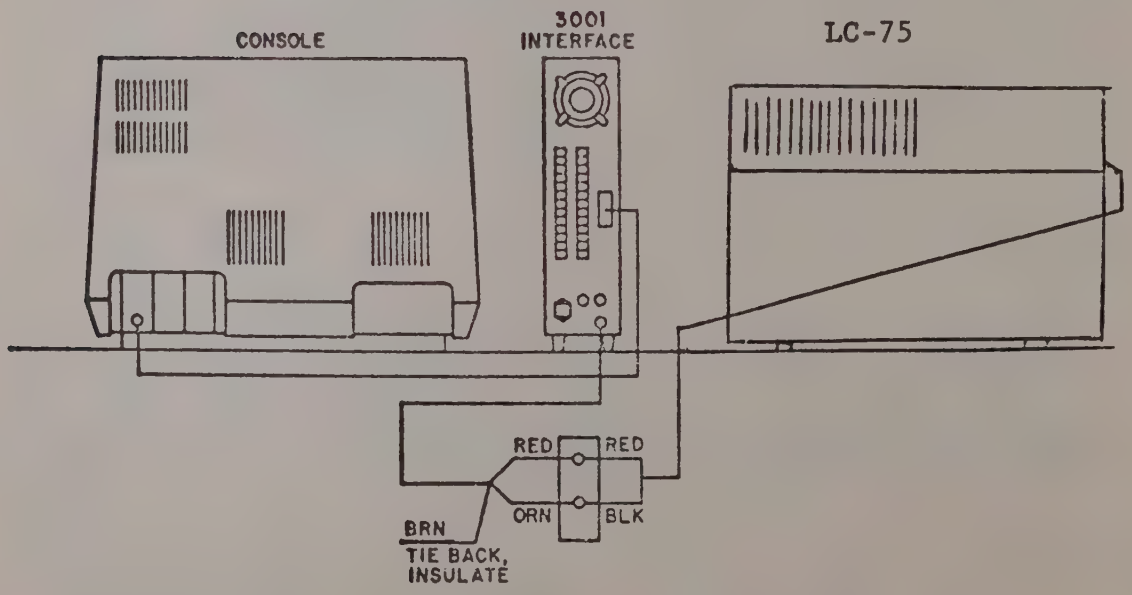


Fig. 2-4 - Console Connection

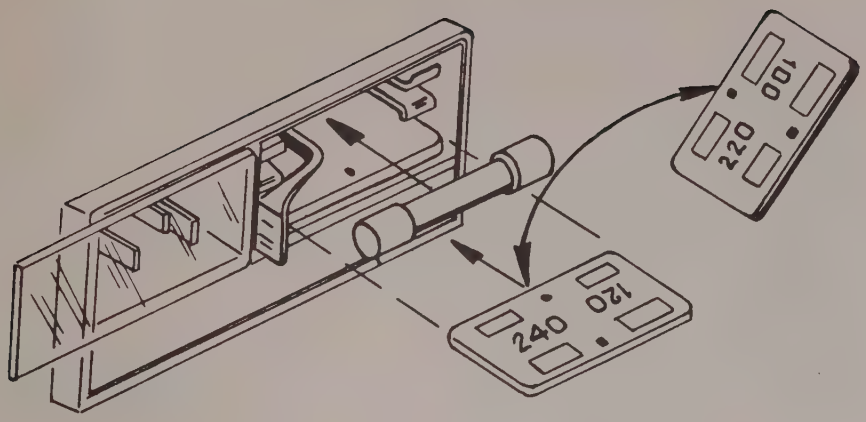


Fig. 2-5 - Voltage Selection

2C,2. Power Connection

First, refer to Figure 2-5 and make certain that the small printed circuit board located behind the line fuse (left side panel) is inserted to correspond to the line voltage available. To select the proper operating voltage range setting, remove the AC line cord from the instrument. Slide the clear plastic cover over the fuse all the way to the left. Rotate the FUSE PULL to the left and remove the fuse. Pull out the printed circuit board located below the fuse and orient the printed circuit board so that the desired voltage setting is legible in accordance with the following:

AC Voltage Range	Legible P.C.B. Setting	Fuse
90-110V	100	1.5A
105-129V	120	1.5A
180-220V	220	.8A
217-258V	240	.8A

Push the printed circuit board back into the slot and rotate the FUSE PULL back into the normal position and make certain that the proper fuse is inserted. Use either a 1.5A (P-E part no. 998-1624) or a 0.8A (P-E part no. 998-1762) fuse depending on the line voltage. Note that both fuses are provided in the Spares Kit. Use only the proper Slo-Blo fuse.

With the cover down and secured in place and the POWER switch OFF, connect the line cord to a properly-grounded AC outlet.

Important: All components of the system, e.g., LC-75, the recorder, the solvent delivery module, etc., must share a common ground in the electircal supply system.

3 SPECIAL LABORATORY REQUIREMENTS

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3 SPECIAL LABORATORY REQUIREMENTS

3A. SITE PREPARATION

The dimensions of the LC-75 are shown in Figure 3-1. Prepare a sturdy bench location in a clean environment with relatively stable temperature and humidity. The weight of the LC-75 is 18.0 kg (40 lbs.). The bench must support the weight of the detector as well as the weight of other items to be used in the system. Provide space at the rear of the instrument for air circulation. Provide access space to the left of the LC-75.

3B. POWER REQUIREMENTS

The detector requires a grounded 117 VAC (nominal) or 230 VAC (nominal) source. The data processing equipment and other accessories require separate outlets. See the appropriate manuals.

3C. CLEAN AIR SYSTEMS

The detector is fitted with an ozone trap to limit the effects of ozone on the spectroscopic analyses.

Observe care in working with hazardous solvents. (See Sections 9 and 10.)

It can generally be assumed that solvent vapor levels high enough to interfere with the detector may also be hazardous for operators who are continually exposed to them.

Provide a clean air source and an appropriate exhaust system in extreme cases.

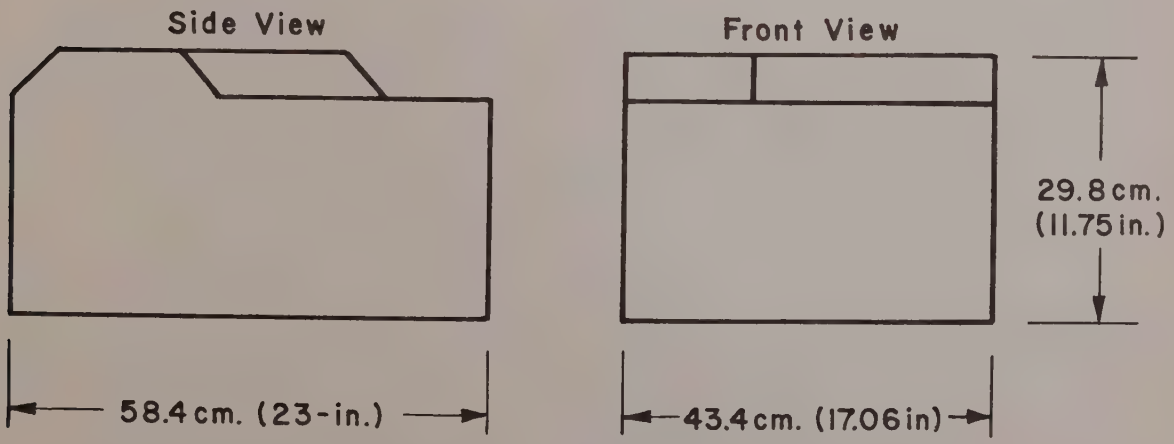

Fig. 3-1 - LC-75 Dimensions

4 PRINCIPLES OF OPERATION

4 PRINCIPLES OF OPERATION

The LC-75 uses a deuterium source (190 to 600 nm).

The amplifier output signal to the recorder is selected by an eleven-step binary attenuator whose most sensitive range is 0.01 absorbance units (A_{1cm}) full scale. Only the output to the recorder is affected by the attenuator as the function of the attenuator is to keep the peaks on scale.

5 PERFORMANCE VERIFICATION PROCEDURE

5 PERFORMANCE VERIFICATION PROCEDURE

This procedure may be used on a routine basis to verify that the detector is perfroming properly.

1) With no flow through the cell, turn the detector POWER ON. Watch the energy meter. After approximately 15 seconds, the deuterium lamp will light and the energy meter will respond.

2) Use the COARSE ZERO control to bring the energy meter into the green region.

3) Use the FINE ZERO to set the recorder baseline.

4) If it is not possible to zero the detector and recorder, replace the column with a dummy column.

5) Pump isopropanol through the system at 6 ml/minute for five minutes. Then disconnect the detector inlet line from the pump outlet and pruge the cell with clean, dry nitrogen at 40 psi (maximum). See Section 2B,3b.

6) Repeat steps 2 and 3 (above). If it is impossible to obtain any energy the lamp must be replaced. (See Section 11.) If it is not possible to zero the detector with maximum energy, call a Perkin-Elmer Service Engineer.

6 SPECIFICATIONS

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6 SPECIFICATIONS

6A. GENERAL

Power Requirements:	75W (max.) at 117V, 60 Hz (nominal) and at 230V, 50 Hz (nominal) (See Section 2.)
Ambient Temperature Range (Operating within specification):	19°C to 30°C
Size:	43.4 cm (17 1/16 in.) wide x 29.8 cm (11 3/4 in.) high x 58.4 cm (23 in.) deep
Weight:	18 kg (40 lbs.)

6B. OPTICAL

Wavelength Range:	190 to 600 nm
Wavelength Accuracy:	±1 nm (approach from low side)
Wavelength Repeatability:	±0.3 nm (approach from low side)
Theoretical Spectral Bandwidth:	3.9 nm at 250 nm 3.6 nm at 600 nm
Stray Radiant Energy:	less than 0.01% at 210 nm
Source Lamps:	deuterium
Wavelength Set:	manual control knob
Wavelength Display:	mechanical readable to 0.1 nm
Order Filter:	Automatic change at 350 and 550 nm nominal
Concentration Range:	Linear within ±0.1% from 0 to 2 A_{1cm} at 275 nm. Within ±5% from 0 to 3 A_{1cm} at 275 nm.
Purge:	Optical paths enclosed; can be purgable with clean dry nitrogen for use in contaminated environments. (See Section 7.)

6C. ELECTRICAL

Energy Readout:	Zero center meter
$Absorbance_{1cm}$ Range:	11-position push-button
Baseline Stability:	less than $\pm$10% per hour at 240 nm and $0.01A_{1cm}$ with a 0.4 Hz, 3-pole Bessel filter. Cell condition: clean and dry with a slight N_2 purge.
Zero Set (at recorder ouput only):	11-position coarse zero; ten-turn potentiometer fine zero
Noise (at 240 nm)	Less than 1.0% p/p of $0.01A_{1cm}$ and 0.4 Hz, 3-pole Bessel filter response. Cell condition: clean and dry with a slight N_2 purge.
Flow Sensitivity (210 nm):	$0.0005A_{1cm}$ from 0 to 1 ml/min. (water)
Outputs	
Recorder:	10 mV for each $Absorbance_{1cm}$ range
Data Output:	0.5V for 5.0 A_{1cm} units
Chart Marker:	pre-set 1.5 mV step on recorder output initiated by external momentary switch closure

6D. CELL

Volume:	8 microliters
Maximum pressure:	14 MPa (2000 psig)
Path Length:	optimized for energy (6 mm) electronically normalized to standard 1cm response

7 OPERATING INSTRUCTIONS

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7 OPERATING INSTRUCTIONS

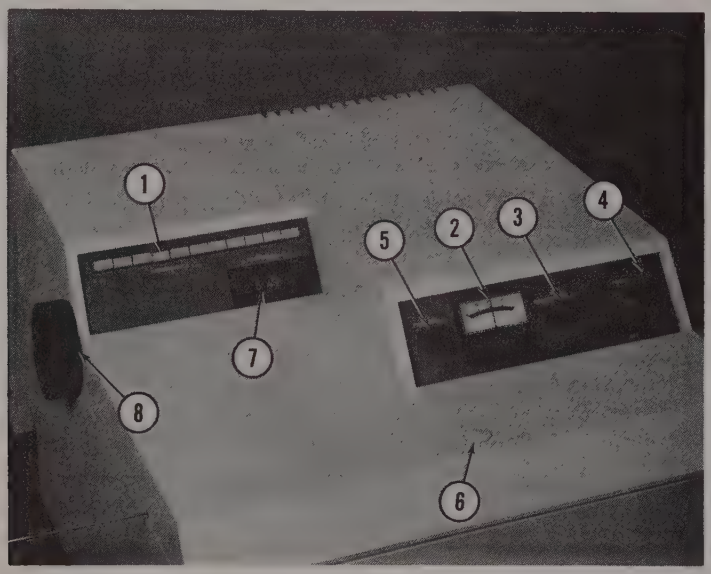
See the end of this Section for a summary of these instructions.

7A. CONTROLS AND INDICATORS

Item numbers correspond to item numbers in Fig. 7-1.

1. $ABSORBANCE_{1cm}$ Range Selector - Eleven-position, push-button binary attenuator adjusts the detector signal to the recorder with the most sensitive setting 0.01 $Absorbance_{1}cm$. One of the attenuator settings must be depressed for the detector to operate.

2. Energy Meter - This zero center meter measures the total lamp energy when the COARSE ZERO is fully counterclockwise. Full scale reading is equal in absorbance units to approximately $5A_{1cm}$ units.



1 $ABSORBANCE_{1cm}$ Range Selector
2 Energy Meter
3 COARSE ZERO
4 POWER Pilot Light
5 FINE ZERO
6 Cap
7 WAVELENGTH Indicator
8 Wavelength Control Knob

Fig. 7-1 - LC-75 Controls and Indicators

3. COARSE ZERO Adjustment Control - Turning this control sets the amplifier to approximately zero. Use this knob to maintain the energy meter in the "green" zone.

4. POWER - Placing the associated switch in the ON (up) position provides power to the LC-75. The POWER pilot light (above the ON-OFF switch) illuminates to verify that the power is on.

5. FINE ZERO Adjustment Control - Turning this control fine sets the recorder baseline and data output.

6. Cap - The screw under this cap secures the detector cover and must be in place for normal operation.

7. WAVELENGTH (nm) Indicator. This wheel indicator provides a three digit and first decimal place readout of the wavelength setting, graduated in 0.2 nm increments readable to 0.1 nm.

8. Wavelength Control Knob - Turning this knob sets the detector wavelength.

> **Important**: 1) Do not force the control knob below the 000.0 nm setting or above the 900.0 nm setting. 2) For repeatability, always approach the desired wavelength from the low side.

7B. DETECTOR OPERATION

7B,1. General Instructions

> **Important**: Do not place anything on the top surface of the detector. Also do not permit any solvent to leak into the detector interior.

1) Connect the system as required for the analysis and set up the detector as described in Section 2.

2) Place the POWER switch at the ON (up) position. Allow approximately 30 minutes for the deuterium lamp to warm up.

3) Set the wavelength control to the appropriate value. Never turn the control to a setting below 000.0 or above 900.0. Always approach the desired setting from the low side to ensure repeatability.

4) Use the COARSE ZERO to bring the energy meter to the green region and FINE ZERO control to set the recorder baseline.

5) The ABSORBANCE setting required will depend on the type of recorder in use as well as the concentration of the components. Attenuate the signal as necessary to keep the peaks on scale.

6) Permit the system to equilibrate by running sufficient solvent through the column and detector until pure solvent flow is established. A flat starting baseline on the recorder is essential for repeatable analyses.

7) Inject the sample. (If a Rheodyne 7105 valve injector is in use, place the valve at the fill position before removing the plug. Then inject the sample, replace the plug, and switch the valve to the inject position.)

7B,2. Use of the ABSORBANCE$_{1cm}$ Range Selector

UV detection of a 1 µg sample of material with typical UV absorbing properties will result in useful recorder data if the proper ABSORBANCE$_{1cm}$ switch is depressed. Initially, use a mid-range ABSORBANCE$_{1cm}$ setting to determine sensitivity required. When a 10 mV recorder is used, an ABSORBANCE$_{1cm}$ setting of 1 results in 0.01 absorbance units (A_{1cm}) full scale. Eleven ABSORBANCE$_{1cm}$ switches are provided.

7B,3. Use of Integrators and Data Systems

The use of the P-E Model Sigma Console or another data acquisition device is acceptable to the LC-75 detector.

Connection of the data acquisition device is made at TB1. (See Section 2C,1.)

7B,4. Zero Procedure

Two controls are provided to calibrate the detector output and to adjust the recorder pen to baseline. These are the COARSE and FINE ZERO adjustment controls.

1) Use the COARSE ZERO adjustment control to adjust the energy meter to within 3 divisions of zero (within the green region on the energy meter).

2) Then use the FINE ZERO adjustment control to bring the recorder baseline to zero.

3) Repeat the procedure at another wavelength.

4) If you are unable to zero the detector, perform the wavelength check provided in Section 8.

7C. SHUTDOWN PROCEDURES

7C,1. Long-term Shutdown

1) Drain the solvent delivery system and clean the reservoir(s) of any solvent, such as carbon tetrachloride or tetrohydrofurane, which might form hazardous by-products.

2) Remove buffer salts from the system and flush with water as evaporation over a period of time will leave salt crystals which may form harmful deposits.

3) Carefully store highly flammable solvents in closed containers.

4) Store the system with some solvent in the system components, particularly in the pump(s). The detector may be left dry, but should be flushed prior to storage. A hexane/chloroform mix is suggested for both flushing and storage. If solvents which are immiscible in hexane/chloroform have been used, flushing the system with an intermediate solvent will be necessary.

5) Consult the column instruction sheet for the specific requirements for the column in use.

6) Placed the marked solvent reservoir on the bench to minimize siphoning.

7) Close the system drain valve if one is incorporated.

8) Shut OFF the POWER to all system components.

> **Important**: The detector deuterium source has a finite life. Turn it off when not in use.

7C,2. Short-term Shutdown (Overnights and Weekends)

1) Observe all the precautions outlined in 7D,1 (above) with respect to hazardous solvents and/or those which may form harmful deposits or by-products.

2) Shut OFF the POWER to the pumps and the Model LC-75 Detector.

> **Important**: The detector deuterium source has a finite life. Turn it off when not in use.

3) If a data acquisition device is in use, consult the device instructions. (Powering-down the data system may not be advantageous if Methods and Data files have not been permanently stored.)

7D. OPERATING SUMMARY

1) Connect the INlet line to the column outlet. If unions are used, make certain that they are of the zero-dead-volume-type.

2) Connect the detector signal outputs to the recorder and/or data system. (See Section 2.)

3) Turn ON the LC-75 POWER. Allow 15-30 minutes minimum warm-up times. (Allow approximately 2 hours for maximum sensitivity setting.)

4) Select the LC pumping system flow rate and detector compatible solvent composition. Begin pumping. Make certain that all plumbing connections are leak-free.

5) Allow the flow rate to stabilize. (Observe the pumping system pressure.)

6) Use the detector WAVELENGTH dial to select a wavelength appropriate for the analysis. Make certain that the wavelength chosen is above the UV cutoff for the solvent(s) in use. (If the sample absorbance wavelength is unknown, use 250 nm initially as many compounds absorb at this wavelength.

7) Select the ABSORBANCE$_{1cm}$ setting. (The ABSORBANCE$_{1cm}$ is used to keep the peaks on-scale. A setting of 0.16 is typical.)

8) Observe the baseline until stable. Adjust the signal to 0 to 10% of the recorder scale with the FINE ZERO control.

9) Inject the sample.

   a) If the peaks go off-scale, select a higher number ABSORBANCE$_{1cm}$ setting to make the peaks smaller. (If a data acquisition device (e.g. P-E Model Sigma Console) is in use, peaks may be allowed to go off-scale.)

   b) If the peaks are too small, use a lower number ABSORBANCE$_{1cm}$ setting.

8 WAVELENGTH CALIBRATION CHECK

8 WAVELENGTH CALIBRATION CHECK

1) Replace the column with a dummy column.

2) Pump isopropanol through the system at 6 ml/minute for 5 minutes.

3) Connect a nitrogen purge line to the cell inlet line. Use only clean, dry nitrogen, preferably prepurified or boil-off from liquid for the purge. Switch on the detector POWER and allow at least 30 minutes stabilization time.

4) Set the wavelength to approximately 486.0 nm and the $ABSORBANCE_{1cm}$ selector at X64.

5) Use the COARSE and FINE ZERO controls to bring the recorder pen on scale.

6) Manually scan the wavelengths about 486 nm for peak energy (a peak of approximately 60 divisions). Note the exact reading of the WAVELENGTH indicator.

7) Repeat steps 4 through 6 except start with the wavelength control at approximately 423.2 nm and set the $ABSORBANCE_{1cm}$ at X32. The energy peak observed should be approximately 15 divisions. Note the exact reading of the WAVELENGTH indicator.

8) The energy peak for the first case should have been observed at a setting of 486.0 $\pm$1nm while the peak in the second case should have been observed at 423.2 $\pm$1nm.

9) If the peak is not observed in either case or if the wavelength at which the peak energy is observed deviates from the above, repeat the procedure. If the deviation still occurs, call a Perkin-Elmer Service Engineer.

9 OPERATIONAL PRECUATIONS AND LIMITATIONS

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9 OPERATIONAL PRECAUTIONS AND LIMITATIONS

9A. SOLVENTS

9A,1. Solvent Vapors

Observe care when working with hazardous solvents or solvents which produce hazardous by-products. Remove sources of solvent vapor by covering the beakers, disposing of solvents properly, etc. In extreme cases, provide a clean air source.

Solvent vapor levels high enough to interfere with the detector should be considered hazardous for the operator continuously exposed to them.

9A,2. Solvent Purity

Solvents should be degassed and filtered to below 0.5 micrometer before use. Solvent Filtration Kits 089-0839 (Aqueous) and 089-0841 (Organic) are available. The solvents commonly used in the far UV are: water, acetonitrile, iso-octane (2,2,4-trimethylpentane), heptane, hexane, and pentane. (Methanol and isopropanol from some suppliers may also be suitable.) Users may have to try several sources because UV-absorbing impurity contents vary from supplier to supplier. Generally, "distilled in glass" solvents are of suitable purity.

9B. SHUTDOWN

As the deuterium source has a finite life, shut OFF the POWER to the LC-75 at the end of the day unless it is absolutely necessary to leave the detector on overnight.

10 HAZARDS

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10 HAZARDS

10A. ELECTRICAL

Because the LC-75 Detector contains the means to power a deuterium lamp, high voltage is present at certain locations in the instrument.

> **Caution**: Do not operate the LC-75 with the cover lifted.

10B. SOLVENTS

Observe care in working with solvents which are hazardous in themselves or which produce hazardous by-products.

> **Caution**: Solvent vapor levels high enough to interfere with the detector should be considered hazardous for the operator who is continuously exposed to them.

10C. GROUND PROTECTION

All the components of the system must share a common ground, supplied by a third wire, in the electrical supply circuit. Do not disconnect or alter this third prong on the LC-75 AC line cord.

10D. STORAGE

Never store anything on the top surface of the detector.

11 SERVICE AND MAINTENANCE

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11 SERVICE AND MAINTENANCE

11A. GENERAL PRECAUTIONS

1) Switch the POWER OFF, and unplug the AC line cord whenever it is necessary to lift the LC-75 cover.

> **Warning**: Do not operate the LC-75 when the cover is lifted.

2) Whenever the cover is lifted and the lamp is on, wear eye protection.

> **Warning**: Never look directly at the lamp when it is on. Wear suitable eye protection in the form of glass safety glasses to prevent serious and irreversible damage to the eyes when servicing the deuterium lamp.

3) Allow the lamp to cool before performing service. Wear thermal protective gloves as necessary to avoid burning the hands.

11B. TROUBLESHOOTING

When detector response and performance changes suddenly, for no apparent reason, the following sequence may be used to narrow the number of possible causes.

> **Note**: Make certain that power is supplied to LC-75. Make certain that the POWER switch is in the ON position, and that the AC line cord is plugged into a working outlet and that the fuses are proper and in good condition.

1) Verify that the deuterium lamp wires are securely connected (refer to Section 11D).

2) Make certain that the deuterium lamp is aligned properly (see Section 11E).

3) Make certain that the cell windows and cell are clean (see Section 11F).

4) Verify that the cell is aligned (see Section 11G).

5) Consult the Troubleshooting Chart given in Table 11-1.

TABLE 11-1 TROUBLESHOOTING CHART

OBSERVATION	PROBABLE CAUSE	REMEDY
No power to detector.	1. Fuse F101 is blow.	1. Replace the fuse with an appropriate one (see Section 11C).
	2. High voltage fuse F102 is blown.	2. Replace the fuse with an appropriate one (see Section 11C).
	3. Low voltage power supply board is faulty.	3. Call a P-E Service Engineer.
Deuterium lamp does not light.	1. Deuterium lamp wires are loose.	1. Turn OFF the POWER, unplug the LC-75 and reconnect the wires (Fig. 11-2).
	2. Deuterium lamp is faulty.	2. Replace the lamp and align it (Sections 11D and 11E).
	3. Lamp driver board is faulty.	3. Call a P-E Service Engineer to replace the lamp driver board.
No detector response.	1. Deuterium lamp is out of position.	1. Align the deuterium lamp (Section 11E).
	2. Foreign object in the sample compartment.	2. Clean the sample cell (Section 11F).
	3. Cell assembly is out of position.	3. Realign the cell assembly (Section 11G).
	4. Wavelength setting is below cutoff wavelength of the solvent.	4. Change to appropriate wavelength.
Low response and 0A cannot be set.	1. Lamp mirror is slightly out of position.	1. Align the lamp (Section 11E).
	2. Foreign object in the sample compartment.	2. Clean the sample cell (Section 11F).
	3. Deuterium lamp is badly darkened.	3. Replace the lamp and align the new one. (Sections 11D and 11E.)
	4. Sample compartment quartz windows are dirty.	4. Clean the quartz window (Section 11F).
	5. Signal amplifiers may require service.	5. Call a P-E Service Engineer.
Detector response is unsteady regardless of the position of the controls.		
1. Deuterium lamp flickers.	1a. Deuterium lamp wire connections are loose.	1a. Reinstall the lamp and realign. (Sections 11D and 11E)
	1b. Deuterium lamp is faulty.	1b. Replace the lamp and align the new one.
2. Deuterium lamp is properly lit and aligned properly.	2a. Solvent is interferring with detector response.	2a. Remove source of solvent interference (Section 9).
	2b. Cell holder is loose.	2b. Tighten cell holder.
	2c. Excessive line voltage fluctuation.	2c. Check line voltage.
	2d. Sample is unstable.	2d. Examine sample for change due to degradation, fading, settling of precipitate, etc. Clean the cell.
Indications are incorrect		
1. Successive indications on the same material are not reproducible.	1. False chemical standard.	1. Check sample for change due to degradation, fading, settling of precipitate, etc.
2. Indications do not agree with previous determinations.	2. Change in chemicals or technique from previous runs.	2. Use the same technique.
3. Calibration is off.	3. Wavelength calibration is off.	3. Check wavelength calibration (Section 11H).

11C. FUSE REPLACEMENT

11C,1. Line Fuse F101

To replace fuse F101 on the AC input plate, see Figure 11-1 and do the following:

1) Turn the POWER switch OFF.

2) Unplug the AC line cord.

3) Open the fuse holder on the left side of the LC-75.

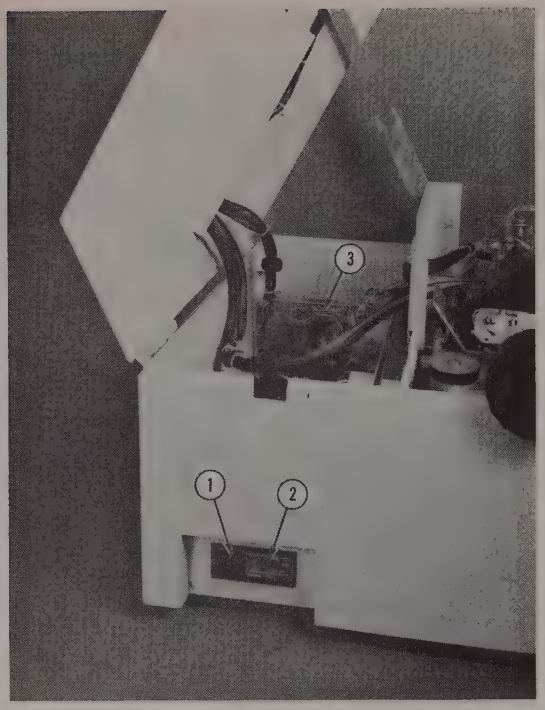
4) If the fuse is blown, remove it and discard it.

5) Place a new fuse in the fuse holder. Use either a 1.5A fuse (P-E part no. 998-1624) for a 117V unit or a 0.8A fuse (P-E part no. 998-1762) for a 234V unit. Use only slo-blo fuses.

6) Close the fuse holder and fuse in the panel.

7) Plug in the AC line cord.

8) Turn the POWER ON.



1 Line Cord Connector
2 F101 Line Fuse
3 F102 HV Fuse

Fig. 11-1 - Fuse Location Details

11C,2. Deuterium Lamp High Voltage Fuse F102

1) See Figure 11-1. With the cover lifted and the detector disconnected from line power, locate the high voltage fuse.

2) Remove the fuse from the fuse holder (remove the protective shield if necessary) and replace it with a 0.5A fuse (P-E part no. 998-1622). Replace the cover and restore power.

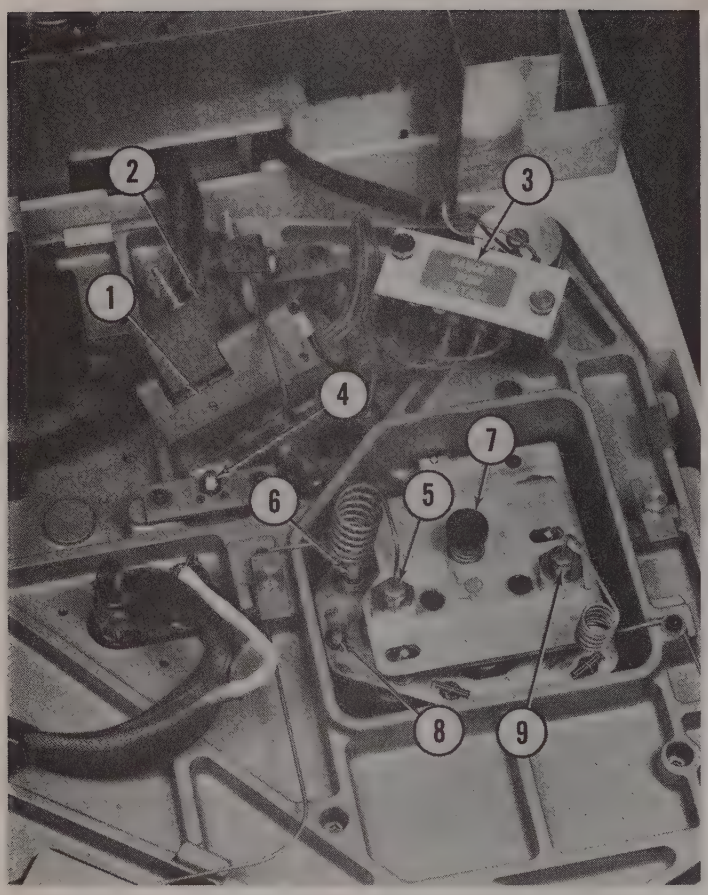
11D. REPLACING THE DEUTERIUM LAMP

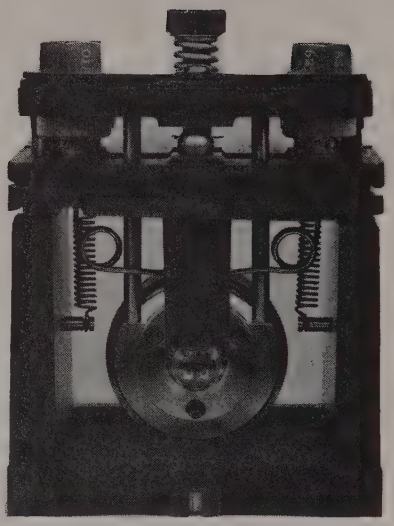
Replace the lamp as follows:

1) Shut OFF the POWER. Unplug the AC line cord. Remove the detector cover.

2) Remove the guard from the lamp terminal strip.

3) See Fig. 11-2 and disconnect the lamp leads. Wear thermal protective gloves to protect your hand from burns or wait for the lamp to cool.





DETAIL OF CELL

1 Lamp
2 Lamp Linear Adjustment
3 Lamp Terminal Strip
4 Lamp Tilt
5 Solvent OUTlet Fitting
6 Cell Horizontal Adjustment
7 Large Thumbscrew
8 Cell Vertical Adjustment
9 Solvent INlet Fitting

Fig. 11-2 - Detector Interior

4) Press down on the front end of the lamp lever to release it. Swing the lever to the back of the LC-75 so that it frees the lamp and lamp bracket. (See Figure 11-3.)

5) Lift the lamp and the magnet lamp bracket from the detector. Do not lose the ozone seal gasket. (See Figure 11-4.)

6) Separate the magnet bracket (with the magnet attached) from the lamp.

7) Discard the old lamp. Replace the lamp with a new deuterium source (P–E part no. 271-1340).

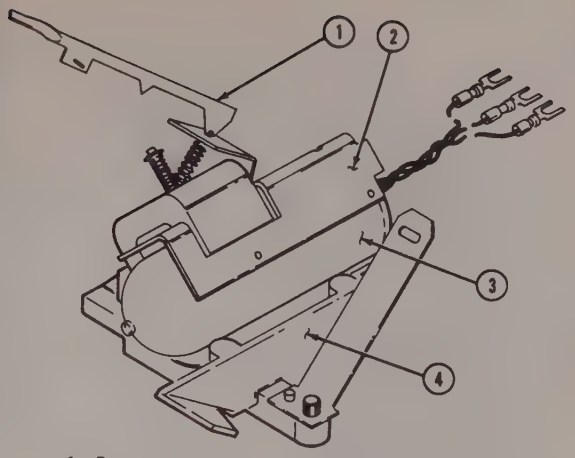
8) DO NOT TOUCH the lamp window itself. Making certain that the ozone seal gasket (P-E part no. 271-1277) is in place around the lamp window and place the prepared lamp in the lamp holder. Hold the lamp in position and place the magnet bracket and magnet assembly over the lamp as shown in Figure 11–3. The end stop on the bracket should rest against the bottom of the source.

9) Using the center notch on the lamp lever to further locate the bracket as shown in Figure 11-5, press down on the lever to secure the pin on the lamp lever in the hole on the lever positioning clip.

10) Note the colors on the deuterium lamp terminal strip screws and connect the lamp wires to the deuterium lamp terminal strip in the following manner.

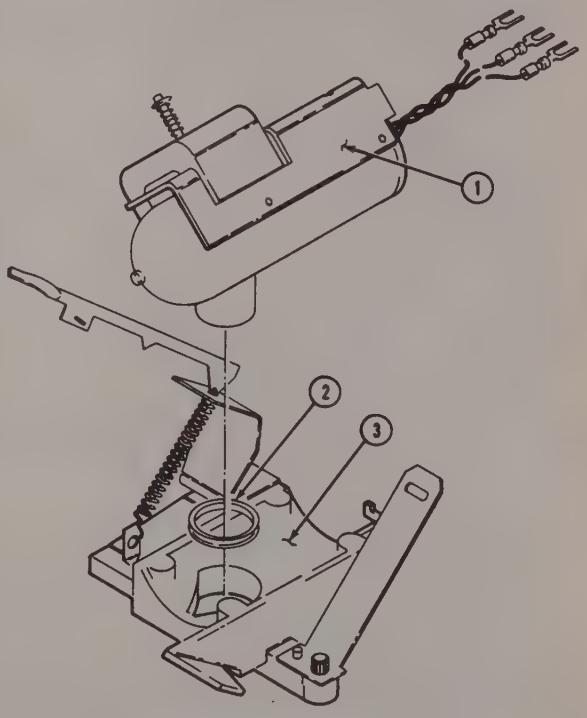
Wire Color	Terminal Position
BLU	3
RED	4
BLU	5

11) Replace the terminal strip guard and the terminal strip cover.



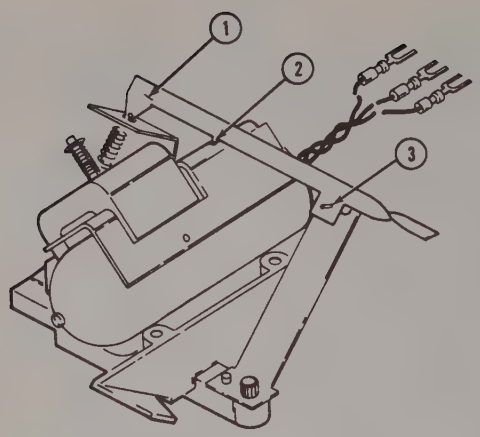
1 Lever
2 Magnet and Lamp Bracket Assembly
3 Deuterium Source
4 Lamp Holder

Fig. 11-3 - Releasing the Lamp Lever



1 Lamp and Magnet Bracket
2 Ozone Seal Gasket, 271-1277
3 Lamp Holder

Fig. 11-4 - Removing the Lamp



1 Lever
2 Center Notch
3 Pin

Fig. 11-5 - Securing the Lamp

12) Plug in the AC line cord. Align the deuterium lamp as described in Section 11E.

11E. ALIGNMENT OF THE DEUTERIUM LAMP

Align the deuterium lamp whenever the lamp has been disturbed or replaced. Make certain that the recorder is connected and operating. This procedure must be performed in a dimly-lit room.

1) Turn the POWER switch ON. Make certain that the solvent flow through the cell is stopped.

2) Wear suitable eye protection in the form of glass safety glasses to prevent damage to the eyes and thermal protective gloves to prevent burns.

3) Set the WAVELENGTH control at 240 nm. Set the ABSORBANCE$_{1cm}$ selector at X8. Zero the LC-75 and the recorder. Set the recorder baseline at 50 divisions.

4) Wait 30 minutes to allow the lamp to warm up.

5) Only use the Allen wrench provided with the detector to adjust the lamp mount in a linear direction (see Fig. 11-2) until maximum energy is observed on the energy meter and the recorder.

6) Then adjust the tilt until the energy meter is at its maximum and the recorder pen indicates minimum absorbance.

7) Align the cell (see Section 11G).

11F. REMOVING THE SAMPLING CELL FOR CLEANING

If it becomes impossible to balance the detector with known clean solvents in the cell, or if baseline noise is experienced, the cell should be examined for cleanliness. If the windows appear to be clean, the problem may result from the presence of a "bubble" of insoluble liquid trapped in the cell (such as hexane in a water system) which can be flushed out with a mutually misicible solvent such as isopropanol. This can easily be determined by proceeding only through step 4, below, then flushing the cell and replacing it as described in steps 15 and 16. See the LC Solvent Polarity and Property Scale in Appendix I.

To remove the sampling cell, proceed as follows:

1) Turn the POWER OFF. Disconnect the AC line cord. Wait for the cell pressure to reach zero. Removing the securing screw blanking cap and the screw and lift the detector cover.

2) Disconnect the inlet and outlet lines from the cell.

3) Loosen the knurled thumbscrew (see Fig. 11-2) on the top of the cell.

4) Lift the sampling cell straight out of the detector. Inspect the cell windows for obvious problems such as bubbles, cracks, or dirt. If further disassembly is required, proceed to step 5 (see Fig. 11-2).

5) Lay the sampling cell down so that the setscrew heads in the cell body are facing up.

6) Unscrew the three #4-40 x 1/2-in. socket head screws which secure the cell retaining rings to the cell and body, and remove the ring that is facing up.

7) Remove the three spring disk washers and note that the direction of the disk contour alternates from disk to disk. When the cell is reassembled, the disks must be replaced in the same alternating order.

8) Remove the window holder.

9) Remove the quartz window.

10) Remove the PTFE window seal that may be stuck to either the window or to the counterbore in the window holder.

11) Turn the cell body over.

12) Remove the other retaining ring and repeat steps 7, 8, and 10.

13) Wipe the cell window with a soft cotton cloth soaked in chloroform, ethanol, or other suitable solvent.

14) Reassemble the cell with new PTFE seals and disk washers. Make sure the contour of the disk washers alternates from washer to washer. Place the first washer in the recess with the outer rim down and the curved portion facing up.

15) Flush out the reassembled cell with distilled water, or other suitable solvent.

16) Place the sample cell back in the detector and tighten the knurled thumbscrew (see Fig. 11-2) on top of the cell.

17) Reconnect the inlet and outlet tubing.

18) Close the detector cover and replace the securing screw and cap.

11G. MICROCELL RE-INSTALLATION AND ALIGNMENT

Perform the following alignment procedure whenever the lamp has been removed or replaced. Do the procedure in a room which is dimly lit.

It is not necessary to realign the cell when the sampling cell alone has been removed. (See Section 11F.)

1) Replace the sampling cell if it has been removed. Tighten the thumbscrew which secures the cell in the interface.

2) Reconnect the solvent lines. The INlet fitting should be toward the right of the instrument. Connect the nitrogen purge line to the sample cell at 40 psi. (See Section 2B,3b.)

3) Plug in the AC line cord if this has been disconnected. Turn ON the POWER switch. Set the WAVELENGTH control to 240 nm. Set the ABSORBANCE$_{1cm}$ selector at X8. Wear glass safety glasses to prevent damage to the eyes. Zero the recorder and LC-75 to recorder 50 divisions.

4) Remove the cell cover. Change the WAVELENGTH control to 190 nm. Keep the ABSORBANCE$_{1cm}$ selector at X8. Refer to Figure 11-2 and insert a 3/32-in. Allen wrench (P-E part no. 990-7271) in the horizontal adjustment hexhead socket. Be sure the wrench is inserted "straight". Observe the recorder pen and the energy meter. Make minute adjustments by rotating the wrench and note if the energy level goes up or down. The object is to obtain the minimum amount of absorbance and maximum energy possible. Increasing energy drives the pen down scale opposite to peak deflection.

5) At the same time adjust the vertical screw until maximum energy is obtained. Try to make adjustments by rotating both screws rather than by moving one screw a large amount. Try to avoid reaching the limits of screw travel.

6) Replace the cell cover when the cell alignment is complete. Replace the cover, securing screw, and blanking cap.

11H. WAVELENGTH RANGE CHECK

This procedure uses the deuterium lamp to check the wavelength calibration. Do this procedure in a dimly-lit room.

1) Place the POWER switch in the ON position. Allow the lamp to warm up until the baseline is stable (approximately 30 minutes). Note that the cell must be clean and dry with a slight nitrogen purge.

> **Warning**: Wear suitable eye protection (glass safety glasses) and do not look directly at the lighted lamp.

2) Set the wavelength to 240 nm and the ABSORBANCE$_{1cm}$ selector at X8. Turn the COARSE ZERO control all the way counterclockwise and then rotate the control three detents in the clockwise direction. Zero the instrument to Recorder Zero with the FINE ZERO control.

3) Set the WAVELENGTH to 190 nm. Rotate the FINE ZERO control to achieve Recorder Zero balance. There should be enough energy to achieve balance.

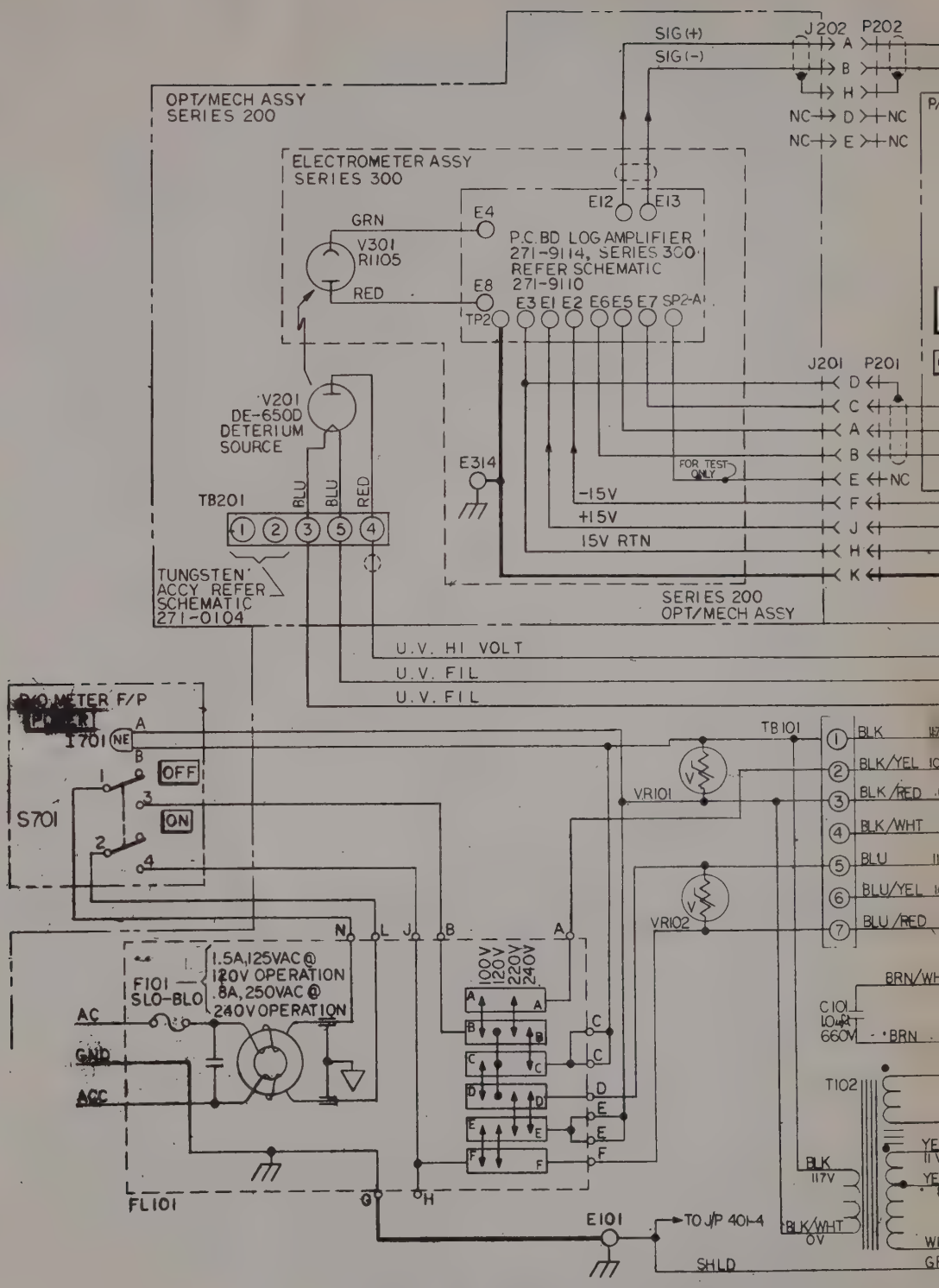
4) Set the WAVELENGTH TO 600 nm, and balance to zero with the FINE ZERO control. Again, there should be enough energy to achieve balance.

5) If this does not occur, realign the lamp and realign the cell. If these do not correct the situation, replace the lamp and realign the lamp and the cell.

11I. REFERENCE SCHEMATICS AND DIAGRAMS

Although service of the LC-75 detector should be entrusted to a Perkin-Elmer Service representative, the following listed diagrams are included for their reference value.

Reference P.C.B./Assy.	Description	Figure No.
LC-75 Final Assembly	Interconnection Diagram, 271-0102D	11-6
LC-75 Final Assembly	Wiring Diagram, 271-0103E	11-7
271-9114, Log Amplifier 271-9153, Coarse Adjust	Amplifier Schematic, 271-9110D	11-8
271-9072, Interface 271-9143, Attenuator	Interface Schematic, 271-9070D	11-9
271-9124, UV Driver	Power Supply/Driver Schematic, 271-9120D	11-10


OPT/MECH ASSY
SERIES 200
ELECTROMETER ASSY
SERIES 300
SIG (+)
SIG (-)
J202 P202
NC D NC
NC E NC
E12
E13
E4
GRN
V301
R1105
RED
E8
P.C. BD LOG AMPLIFIER
271-9114, SERIES 300
REFER SCHEMATIC
271-9110
E3 E1 E2 E6 E5 E7 SP2-A1
TP2
V201
DE-650D
DETERIUM
SOURCE
E314
TB201
BLU
BLU
RED
TUNGSTEN
ACCY REFER
SCHEMATIC
271-0104
J201 P201
D
C
A
B
E NC
F
J
H
K
FOR TEST ONLY
-15V
+15V
15V RTN
SERIES 200
OPT/MECH ASSY
U.V. HI VOLT
U.V. FIL
U.V. FIL
S701
OFF
ON
V.R101
V.R102
TB101
BLK
BLK/YEL
BLK/RED
BLK/WHT
BLU
BLU/YEL
BLU/RED
BRN/WH
C101
660V
BRN
T102
BLK
117V
BLK/WHT
0V
1.5A, 125VAC @
120V OPERATION
.8A, 250VAC @
240V OPERATION
F101
SLO-BLO
AC
GND
ACC
100V
120V
220V
240V
FL101
E101
TO J/P 401-4
SHLD
POWER SUPPLY ASSY

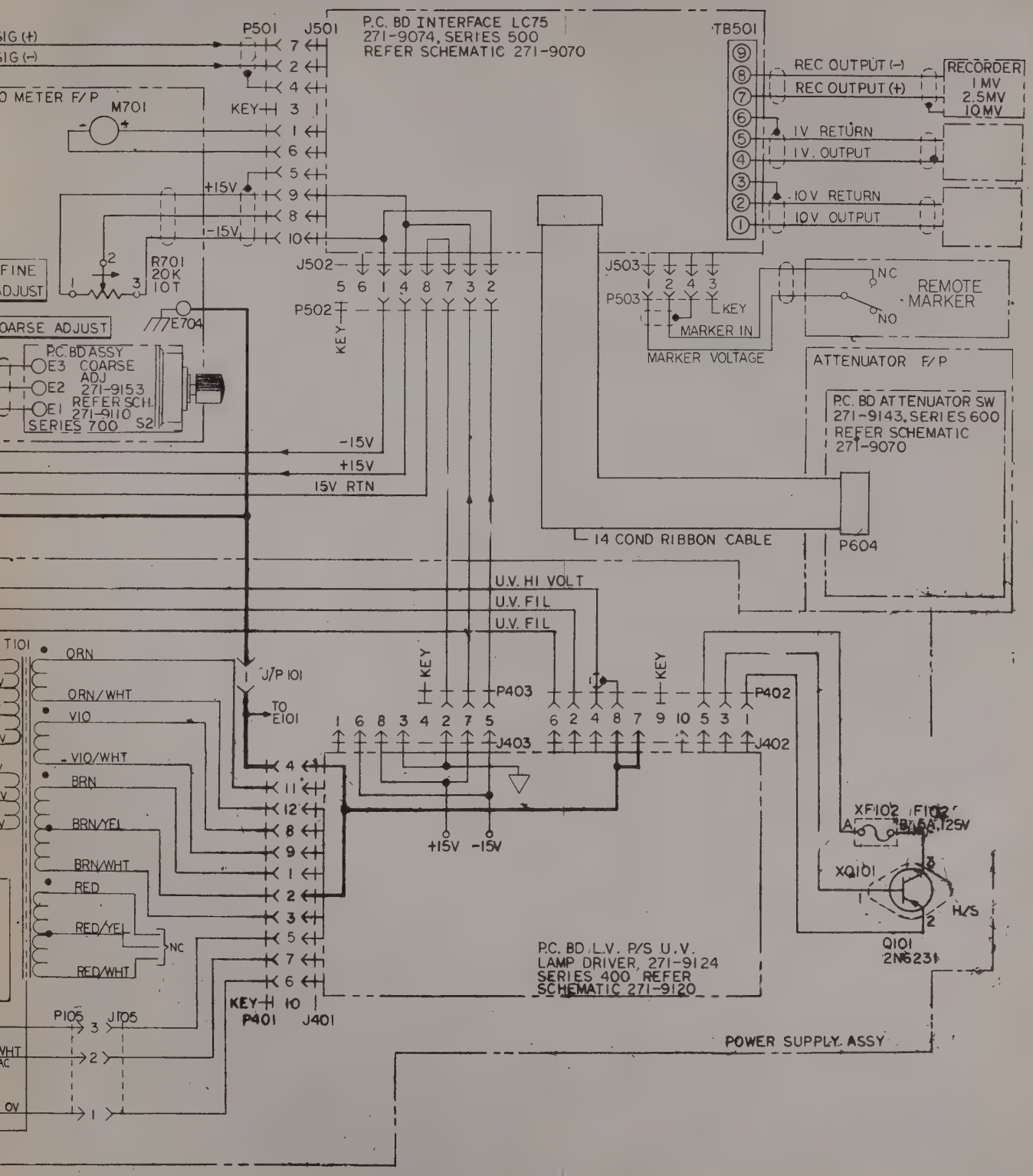


Figure 11-6 - Interconnection Diagram, 271-0102D

OPT/MECH ASSY (TOP)
P/O HARNESS ASSY
271-0254
P201
P202
J201
J202
P502-4 BLU 12
P502-3 BLK 11
P501-1 GRN/YEL 8
P502-1 W/BLU 10
SHLD BLK 9
E703 RED 7
E701 BRN 6
E702 ORN 5
P501-7 RED 1
SHLD BLK 4
P501-2 BLK 2
E805 ORN
E301 BLU
E308 BLK
E314 GRN/YEL
E302 W/BLU
SP-302-A YEL
E803 BLK
E807 RED
E306 BRN
* TEST POSITION ONLY
E312 RED 1
SHLD BLK 3
E313 BLK 2
HARNESS ASSY
271-0259
OPT/MECH ASSY (BOTTOM)
ELECTROMETER ASSY
FRONT
LC 75
J201-A WHT 7
J201-B BRN 6
J201-J BLU 8
J201-D BLK 4
J201-F W/BLU 10
J201-C RED 5
J201-E YEL 12
E314 GRN/YEL 13
E306
E305
E301
E308
E307
E304
SP-302-A
E308
E312
E313
E314
J202-8 BLK 2
J202-A RED 1
PC BD ASSY-
LOG AMPLIFIER
271-9114
REF SCHEMATIC
271-9110
V301
GRN
PHOTO
DETECTOR
P.C. BD ASSY- L.V. PS/
U.V. LAMP DRIVE
271-9124
P502-3 BLU 15
P502-2 W/BLU 14
KEY
P502-7 BLK 13
P403
KEY
SHLD BLK 2
TB201-3 W/BLU 3
XF102-A ORN 7
TB201-4 VIO 1
XQ101-1 BRN 6
TB201-5 BLU 4
XQ101-2 YEL 5
P402
REF SCHEMATIC 271-9120
P401
V201
U.V. SOURCE
BLU
RED
BLU
P402-5 BLU 4
P402-4 VIO 1
P402-6 W/BLU 3
TB201
(TOP) OPT/MECH ASSY

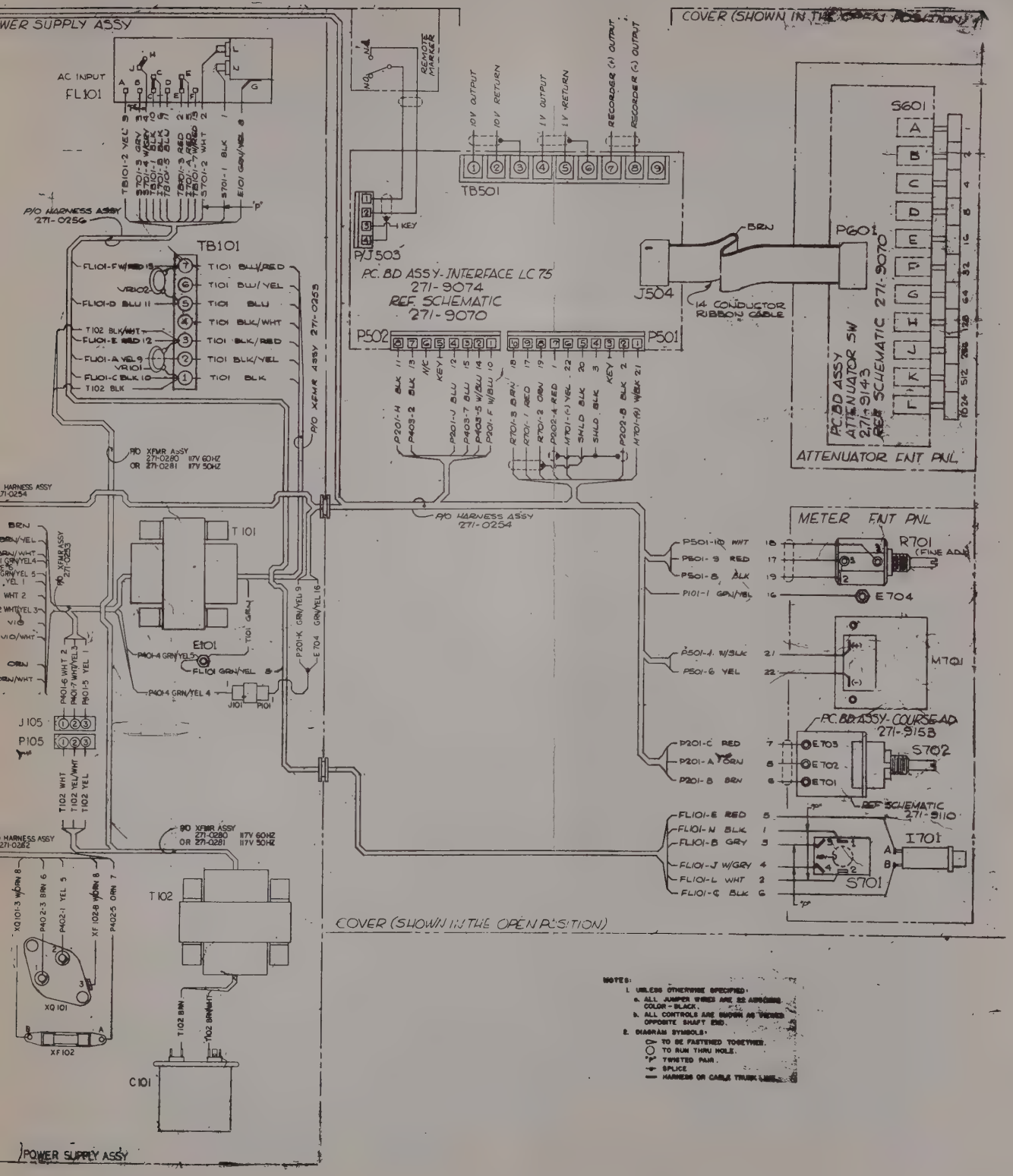
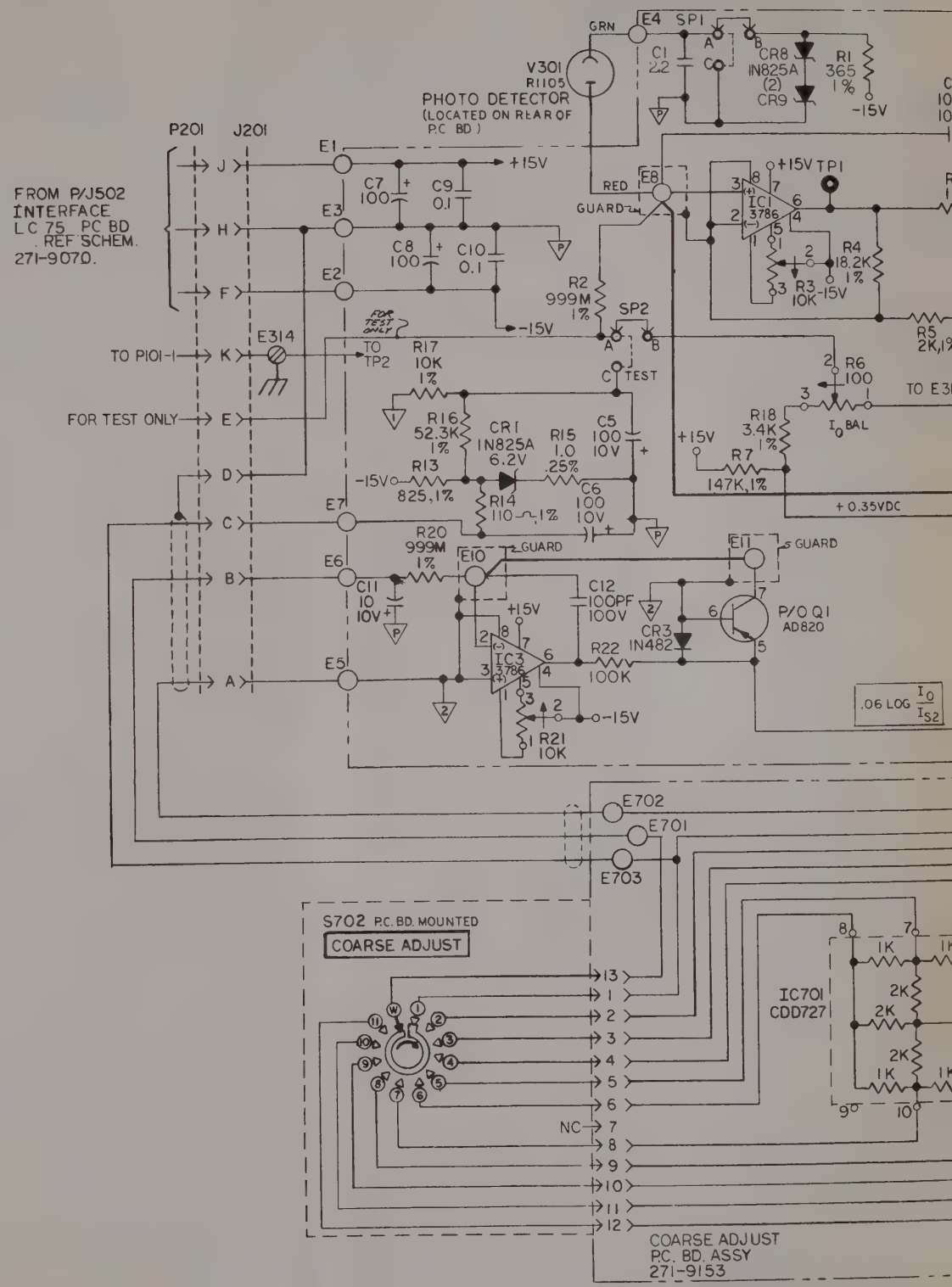
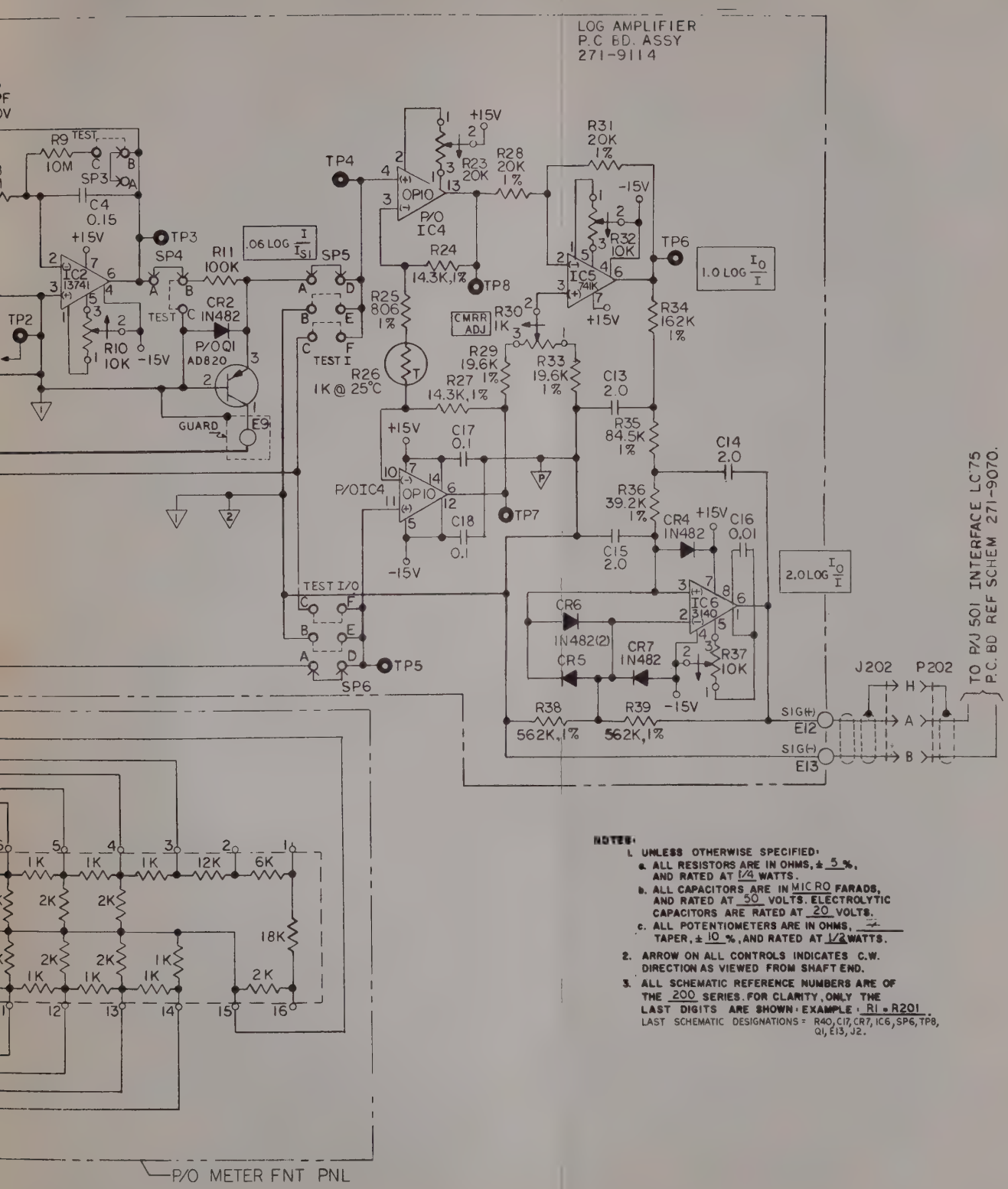


Figure 11-7 - Wiring Diagram, 271-0103E

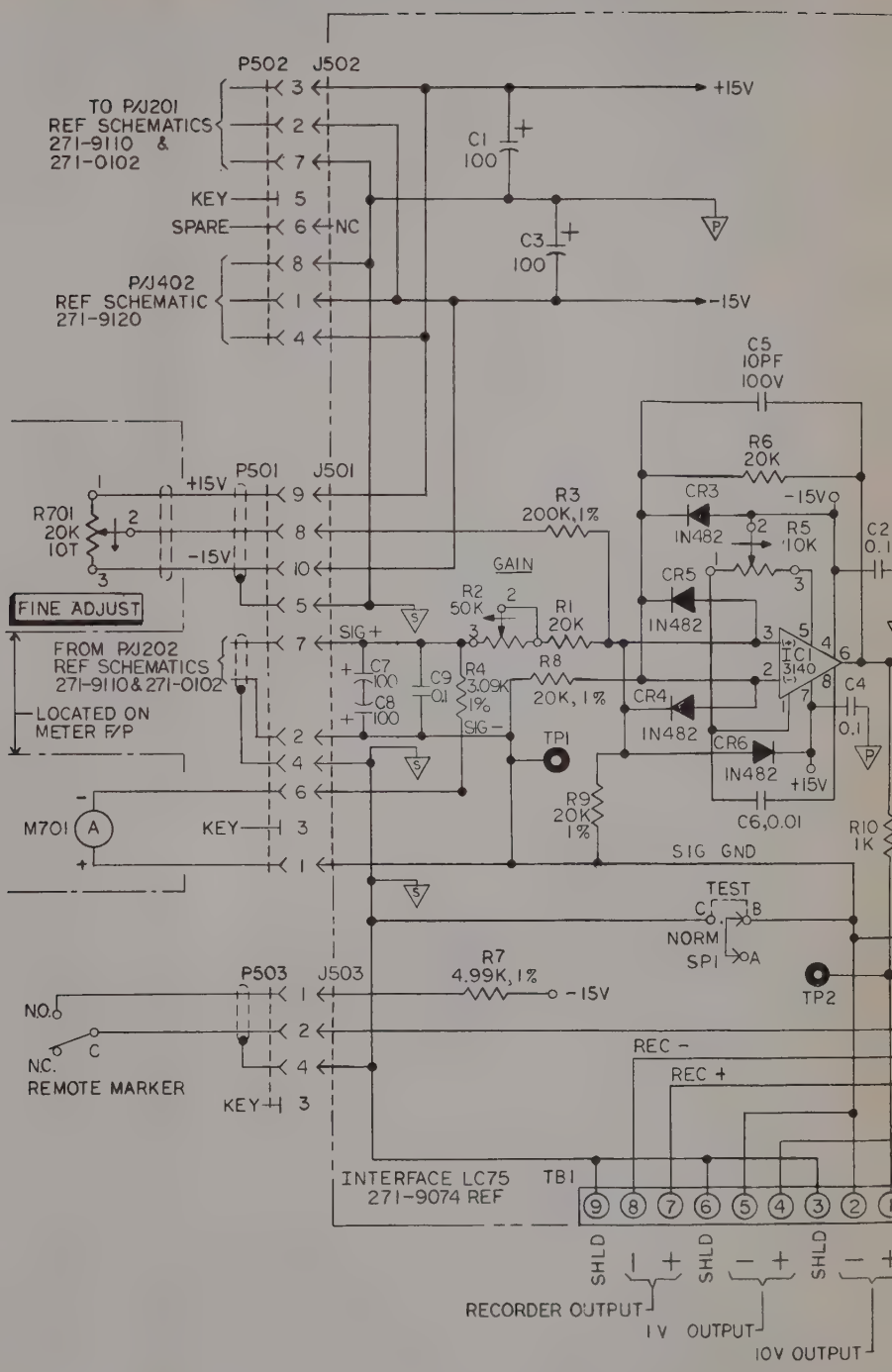

GRN
E4
SP1
V301
R1105
PHOTO DETECTOR
(LOCATED ON REAR OF P.C. BD.)
C1
22
CR8
IN825A
(2)
CR9
R1
365
1%
-15V
P201
J201
FROM P/J502
INTERFACE
LC 75 PC BD
REF SCHEM.
271-9070.
E1
+15V
C7
100
C9
0.1
E3
C8
100
C10
0.1
E2
RED
E8
GUARD
+15V
TP1
IC1
3786
R4
18.2K
1%
R3
10K
-15V
R2
999M
1%
SP2
R5
2K,1%
FOR TEST ONLY
E314
TO P101-1
K
TO TP2
R17
10K
1%
TEST
R6
100
TO E3
FOR TEST ONLY
E
R16
52.3K
1%
CR1
IN825A
6.2V
R15
1.0
.25%
C5
100
10V
R18
3.4K
1%
I0 BAL
+15V
R7
147K,1%
D
R13
825,1%
-15V
R14
110
1%
C6
100
10V
+0.35VDC
E7
C
R20
999M
1%
E10
GUARD
E11
GUARD
E6
B
C11
10
10V
C12
100PF
100V
+15V
IC3
3786
CR3
IN482
P/O Q1
AD820
R22
100K
E5
A
.06 LOG I0/IS2
-15V
R21
10K
E702
E701
E703
S702 P.C. BD. MOUNTED
COARSE ADJUST
13
1
2
3
4
5
6
NC
7
8
9
10
11
12
IC701
CDD727
1K
2K
2K
2K
1K
1K
COARSE ADJUST
P.C. BD. ASSY
271-9153



NOTES:

1. UNLESS OTHERWISE SPECIFIED:
   a. ALL RESISTORS ARE IN OHMS, ± 5 %, AND RATED AT 1/4 WATTS.
   b. ALL CAPACITORS ARE IN MICRO FARADS, AND RATED AT 50 VOLTS. ELECTROLYTIC CAPACITORS ARE RATED AT 20 VOLTS.
   c. ALL POTENTIOMETERS ARE IN OHMS, ___ TAPER, ± 10 %, AND RATED AT 1/2 WATTS.
2. ARROW ON ALL CONTROLS INDICATES C.W. DIRECTION AS VIEWED FROM SHAFT END.
3. ALL SCHEMATIC REFERENCE NUMBERS ARE OF THE 200 SERIES. FOR CLARITY, ONLY THE LAST DIGITS ARE SHOWN. EXAMPLE: R1 = R201.
   LAST SCHEMATIC DESIGNATIONS = R40, C17, CR7, IC6, SP6, TP8, Q1, E13, J2.

Figure 11-8 - Amplifier Schematic, 271-9110D


P502 J502
TO PJ201 REF SCHEMATICS 271-9110 & 271-0102
KEY
SPARE
NC
PJ402 REF SCHEMATIC 271-9120
+15V
-15V
C1 100
C3 100
C5 10PF 100V
R6 20K
P501 J501
R701 20K 10T
FINE ADJUST
FROM PJ202 REF SCHEMATICS 271-9110 & 271-0102
LOCATED ON METER F/P
R3 200K, 1%
GAIN
R2 50K
R1 20K
SIG +
SIG -
C7 100
C8 100
C9 0.1
R4 3.09K 1%
R8 20K, 1%
TP1
R9 20K 1%
M701
A
KEY
SIG GND
CR3 IN482
CR4 IN482
CR5 IN482
CR6 IN482
R5 10K
IC1 3140
C4 0.1
C6, 0.01
R10 1K
TEST
NORM
SP1
TP2
P503 J503
R7 4.99K, 1%
N.O.
N.C.
C
REMOTE MARKER
REC -
REC +
INTERFACE LC75 271-9074 REF
TB1
SHLD
RECORDER OUTPUT
1V OUTPUT
10V OUTPUT

NOTES:

1. UNLESS OTHERWISE SPECIFIED:
   a. ALL RESISTORS ARE IN OHMS, ± 5 %, AND RATED AT 1/4 WATTS.
   b. ALL CAPACITORS ARE IN MICRO FARADS, AND RATED AT 50 VOLTS. ELECTROLYTIC CAPACITORS ARE RATED AT 20 VOLTS.
   c. ALL POTENTIOMETERS ARE IN OHMS, —/— TAPER, ± 10 %, AND RATED AT 1/2 WATTS@70°
2. ARROW ON ALL CONTROLS INDICATES C.W. DIRECTION AS VIEWED FROM SHAFT END.
3. ALL SCHEMATIC REFERENCE NUMBERS FOR INTERFACE BD ARE OF THE 500 SERIES. FOR CLARITY, ONLY THE LAST DIGITS ARE SHOWN: EXAMPLE: R1 = R501.
   LAST SCHEMATIC DESIGNATIONS: R10 C6, CR6, IC1, TP2, TB1, J3, SP1.
4. ALL SCHEMATIC REFERENCE NUMBERS FOR ATTENUATOR BD ARE OF THE 600 SERIES. FOR CLARITY, ONLY THE LAST DIGITS ARE SHOWN: EXAMPLE: R1 = R601.
   LAST SCHEMATIC DESIGNATIONS: R3, C2, J1, SP1, S1.

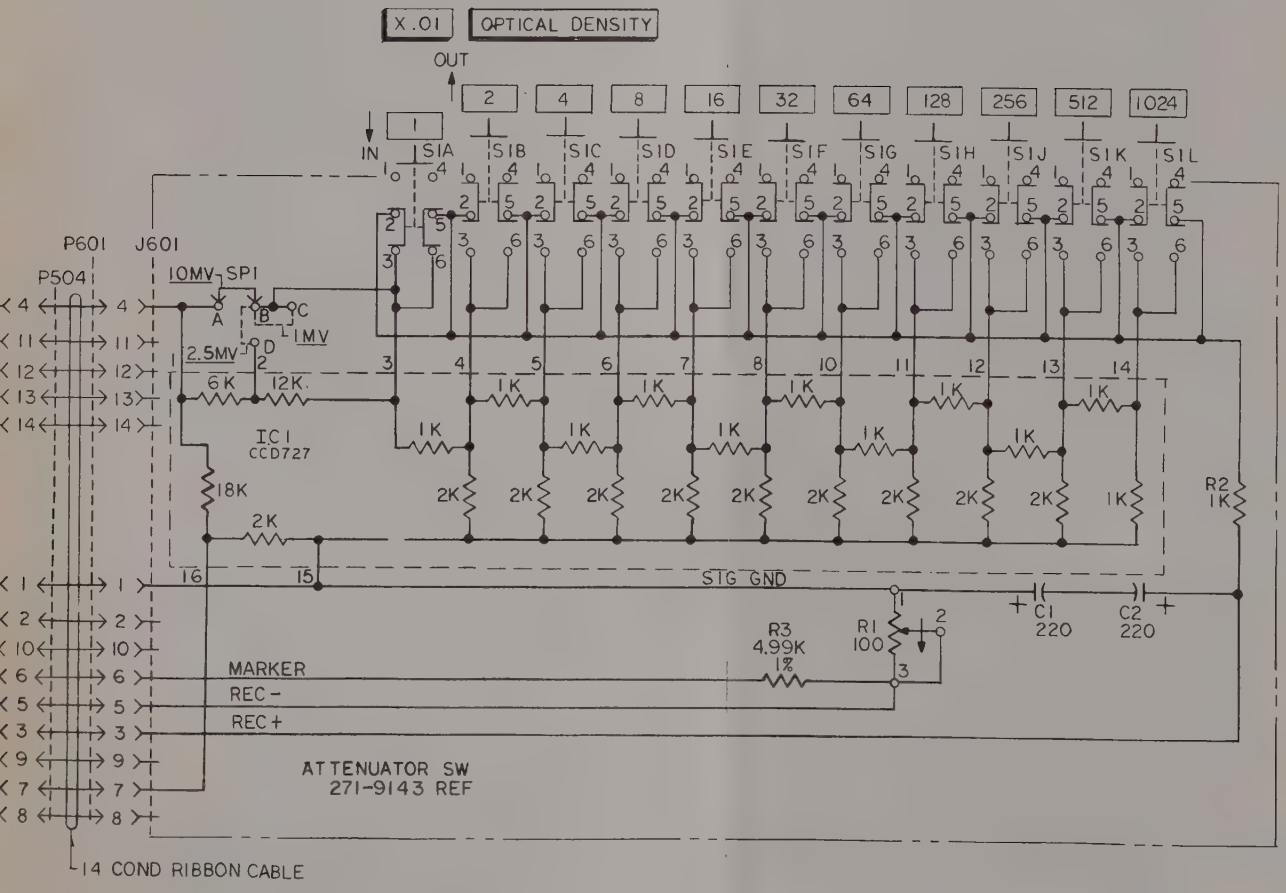
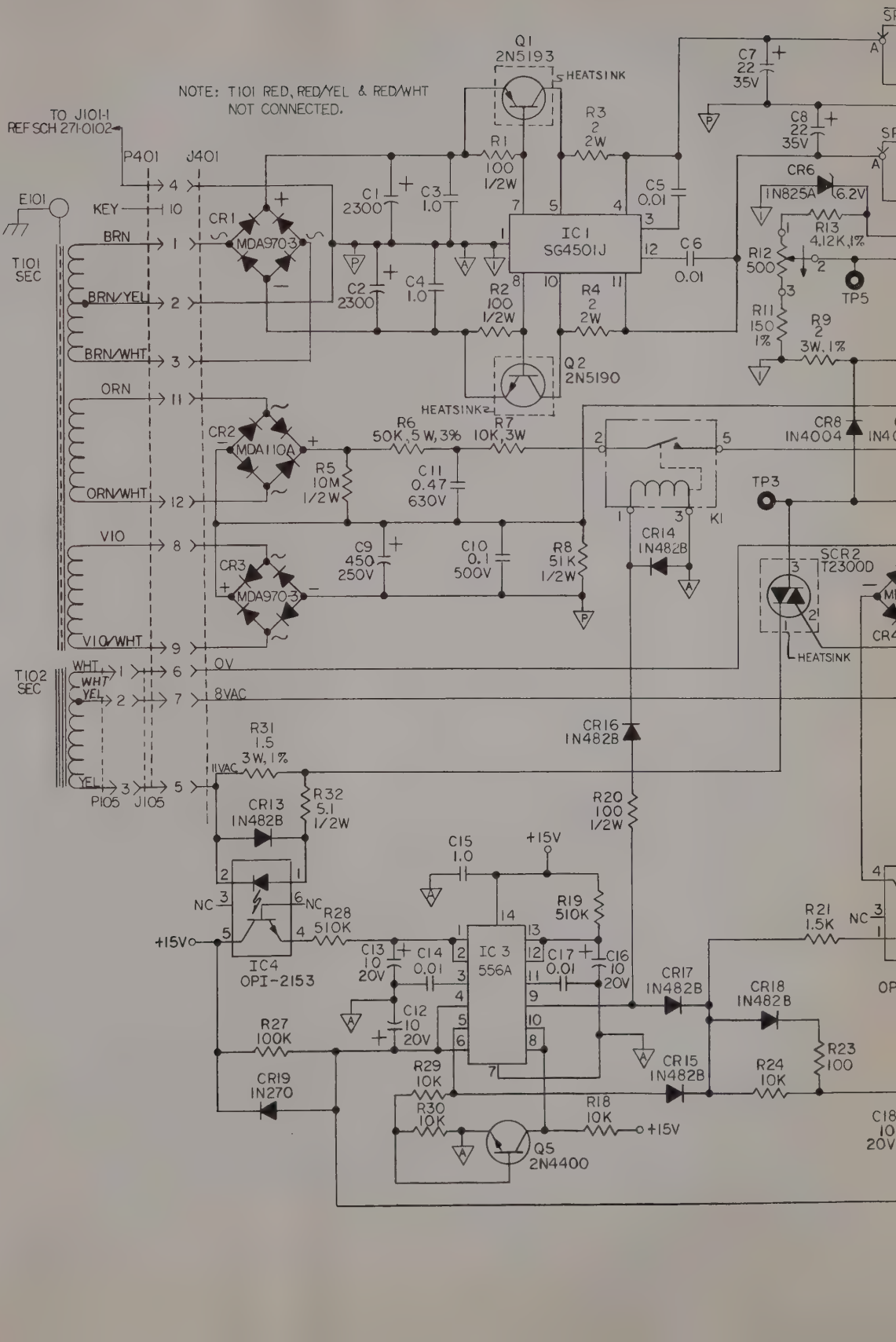
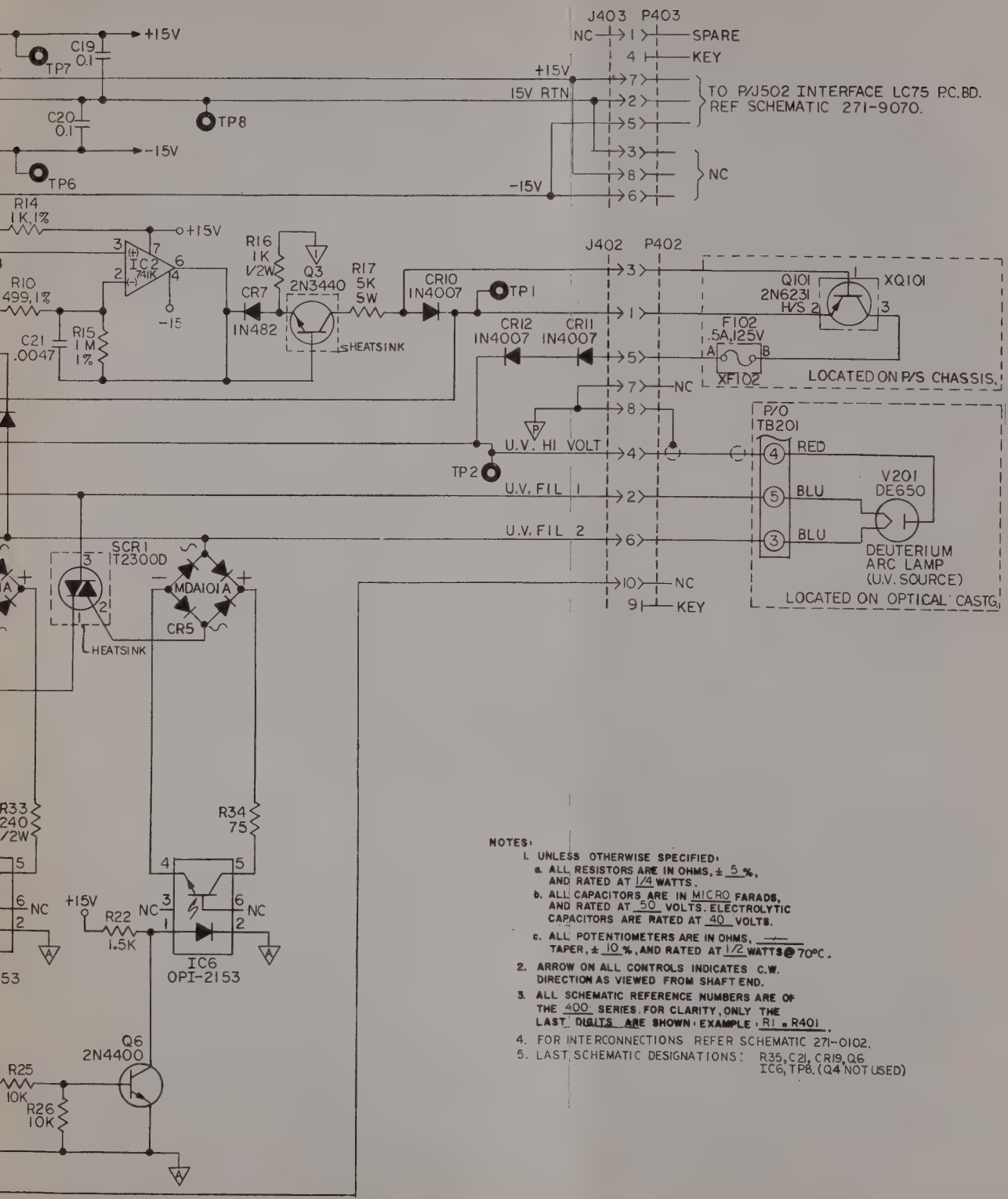


Figure 11-9 - Interface Schematic, 271-9070D


NOTE: T101 RED, RED/YEL & RED/WHT NOT CONNECTED.
TO J101-1 REF SCH 271-0102
P401
J401
E101
KEY
BRN
BRN/YEL
BRN/WHT
ORN
ORN/WHT
VIO
VIO/WHT
T101 SEC
T102 SEC
WHT
WHT
YEL
YEL
P105
J105
CR1 MDA970-3
CR2 MDA110A
CR3 MDA970-3
C1 2300
C2 2300
C3 1.0
C4 1.0
C5 0.01
C6 0.01
C7 22 35V
C8 22 35V
C9 450 250V
C10 0.1 500V
C11 0.47 630V
R1 100 1/2W
R2 100 1/2W
R3 2 2W
R4 2 2W
R5 10M 1/2W
R6 50K, 5W, 3%
R7 10K, 3W
R8 51K 1/2W
R9 2 3W, 1%
R11 150 1%
R12 500
R13 4.12K, 1%
Q1 2N5193
Q2 2N5190
HEATSINK
IC1 SG4501J
CR6 1N825A 6.2V
CR8 1N4004
K1
TP3
TP5
CR14 1N482B
SCR2 T2300D
CR16 1N482B
0V
8VAC
R31 1.5 3W, 1%
11VAC
R32 5.1 1/2W
CR13 1N482B
R20 100 1/2W
C15 1.0
+15V
R19 510K
IC4 OPI-2153
NC
R28 510K
IC 3 556A
C13 10 20V
C14 0.01
C17 0.01
C16 10 20V
CR17 1N482B
CR18 1N482B
R21 1.5K
R23 100
R24 10K
C12 10 20V
R27 100K
CR19 1N270
R29 10K
R30 10K
R18 10K
Q5 2N4400
CR15 1N482B



NOTES:

1. UNLESS OTHERWISE SPECIFIED:
   a. ALL RESISTORS ARE IN OHMS, ± 5 %, AND RATED AT 1/4 WATTS.
   b. ALL CAPACITORS ARE IN MICRO FARADS, AND RATED AT 50 VOLTS. ELECTROLYTIC CAPACITORS ARE RATED AT 40 VOLTS.
   c. ALL POTENTIOMETERS ARE IN OHMS, ___ TAPER, ± 10 %, AND RATED AT 1/2 WATTS @ 70°C.
2. ARROW ON ALL CONTROLS INDICATES C.W. DIRECTION AS VIEWED FROM SHAFT END.
3. ALL SCHEMATIC REFERENCE NUMBERS ARE OF THE 400 SERIES. FOR CLARITY, ONLY THE LAST DIGITS ARE SHOWN. EXAMPLE: R1 = R401.
4. FOR INTERCONNECTIONS REFER SCHEMATIC 271-0102.
5. LAST SCHEMATIC DESIGNATIONS: R35, C21, CR19, Q6 IC6, TP8. (Q4 NOT USED)

Figure 11-10 - Power Supply/Driver Schematic, 271-9120D

12 ACCESSORIES AND OPTIONS

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A.	Accessories	12-1
B.	Warranty Information	12-1

12 ACCESSORIES AND OPTIONS

12A. ACCESSORIES

271-0330* Auto Control, 117V, 50/60 Hz
271-0331* Auto Control, 234V, 50/60 Hz
271-0311 LC-75 Upgrade Kit for Auto Control

12B. WARRANTY INFORMATION

The Seller hereby warrants to the Buyer that the equipment sold hereunder is at the time of shipment to the Buyer from Seller free from defects in material and workmanship. Seller agrees either to repair or replace at Seller's sole option and free of part charge to the Buyer any part or parts of such equipment which under proper and normal condition of use prove to be defective within twelve (12) months from the date of shipment to the Buyer. It is recognized that some parts by their nature may not function one year; therefore, excluded from the foregoing warranty are filaments, energy sources, lamps, graphite tubes, sample cells, holders, burner chambers, nebulizers, along with other parts mentioned in the applicable operating manual. After installation, any realignment, readjustment, recleaning, or recalibration, providing they do not relate to a provem defect in material or workmanship, shall be performed only at Seller's then current rates for service.

The foregoing warranty excludes certain major items or accessories specifically indicated on applicable price lists or quotations, as to which Seller passes to the Buyer whatever warranty is provided to Seller by the manufacturer or the specific warranty indicated by the price list or quotation.

This warranty specifically does not cover loss or damage to equipment which might be sustained in transit to the Buyer's facility, as to which Seller disclaims any responsibility whatsoever.

Seller shall use its best efforts to perform all warranty service hereunder at the Buyer's facility, and as soon after notification by the Buyer of a possible defect as is reasonably practicable; provided, however, that Seller reserves the right to require that the Buyer return the equipment to Seller's production facility, transportation charges prepaid, when necessary to provide proper warranty service.

In lieu of the foregoing, Seller may at any time elect, in its sole discretion, to discharge its warranty by accepting the return of such equipment and refunding the purchase price to the Buyer.

THE FOREGOING WARRANTY AND REMEDY ARE EXCLUSIVE AND EXPRESSLY IN LIEU OF ALL OTHER WARRANTIES EXPRESS OR IMPLIED EITHER IN FACT OR BY OPERATION OF LAW, STATUTORY OR OTHERWISE, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR USE. SELLER NEITHER ASSUMES NOR AUTHORIZES ANY OTHER PERSON TO ASSUME FOR IT ANY OTHER LIABILITY IN CONNECTION WITH THE SALE, INSTALLATION, SERVICE OR USE OF ITS EQUIPMENT. SELLER SHALL HAVE NO LIABILITY WHATSOEVER FOR SPECIAL, CONSEQUENTIAL OR PUNITIVE DAMAGES OF ANY KIND FROM ANY CAUSE ARISING OUT OF THE SALE, INSTALLATION, SERVICE OR USE OF ITS EQUIPMENT.

*Purchased separately from LC-75 Detector. Requires Upgrade Kit 271-0311 which is Service or Factory installed.

APPENDICES

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Appendix I - LC Solvent Polarity and Property Scale	AI-1
Appendix II - Ultraviolet Absorption Bands of Common Chromophores	AII-1
Appendix III - Column + Solvent Selection Guide	AIII-1
Appendix IV - Common LC Calculations	AIV-1
Appendix V - The General Elution Problem and Its Solution	AV-1
Appendix VI - Fundamental Concepts of Liquid Chromatography	AVI-1

APPENDIX I

LC SOLVENT POLARITY AND PROPERTY SCALE

Solvent	Adsorptive Energy	Boiling Point, °C	Viscosity	Refractive Index	UV cutoff/nm
*Hexane	0.00	69	0.33	1.375	210**
*Isoctane	0.01	99	0.3	1.404	210**
Petroleum ether Skellysolve B, etc.	0.01	175-240	0.3		210
n-Decane	0.04	174	0.92	1.412	
Cyclohexane	0.04	81	1.00	1.427	210
Cyclopentane	0.05	49	0.47	1.406	210**
Carbon tetrachloride	0.18	77	0.97	1.466	265
Amyl chloride	0.26	108	0.43	1.413	225
*Butyl chloride	0.26	77	0.47	1.436	220
Xylene	0.26	138-144	0.62-0.81	~1.50	290
*i-Propyl ether	0.28	69	0.37	1.368	220
Toluene	0.29	111	0.59	1.496	285
Chlorobenzene	0.30	132	0.80	1.525	330
Benzene	0.32	80	0.65	1.501	280
*Chloroform	0.40	61	0.57	1.443	245
Methyl-i-butylketone	0.43	117		1.394	330
*Tetrahydrofurane	0.45	65	0.35	1.408	220
Ethylene dichloride	0.49	84	0.79	1.445	230
Methylethylketone	0.51	80	0.3	1.381	330
Acetone	0.56	57	0.32	1.359	330
*Dioxane	0.56	101	1.54	1.422	220
Ethyl acetate	0.58	77	0.45	1.370	260
*Amyl alcohol	0.61	138	4.1	1.410	210
Dimethyl sulfoxide	0.62	189	2.24		
Nitromethane	0.64	101	0.67	1.394	380
*Acetonitrile	0.65	82	0.37	1.344	210**
Pyridine	0.71	115	0.94	1.510	305
*i-propanol, n-propanol	0.82	83	2.3	1.38	210
Ethanol	0.88	79	1.20	1.361	210
*Methanol	0.95	65	0.60	1.329	210
Ethylene glycol	1.11	198	19.9	1.427	210
Acetic acid	Large	118	1.26	1.372	
*Water	Larger	100	1.0	1.333	190**
Salts & Buffer	Very Large	100+			

List of common chromatographic solvents in order of increasing polarity with RI values. From "Principles of Adsorption Chromatography" L.R. Snyder, Dekker.

*Most commonly used with U.V. Detector.
**Extremely pure grades may be used as low as 195 nm.

The Hildebrand solvent scale is a list of common solvents used in liquid Chromatography in order of increasing energy of adsorption on alumina. The values are different, but the order is essentially the same on silica gel as on alumina.

The starting solvent selected for a given separation can be chosen by matching the relative polarity of the solvent to that of the sample. This is done as a first approximation by selecting the solvent to match the most polar functional group on the sample molecule. (eg alcohols of OH, amines for NH_2 etc.) From this attempt the separation can be refined by the following procedure.

1. If the sample appears at the solvent front then the solvent is too polar to allow the adsorbent to retard the sample. Go to a solvent higher up (lower polarity) on the scale.

2. Conversely if the sample does not appear in a reasonable time go to a solvent or solvent blend lower down (higher polarity) on the scale.

Solvent blends are most useful when they are composed of pairs which differ in adsorption energy by no more than 100%. (Example propanol at energy 0.82 and chloroform at energy 0.40)

Solvent gradients should be generated on the same basis of no more than doubling the energy of the starting solvent. The range is more than sufficient for most if not all L C separations.

When changing solvents or when regenerating after a gradient, a minimum of 5 column volumes of the new starting solvent should flow through the system before another sample injection is made.

Notes on solvent miscibility

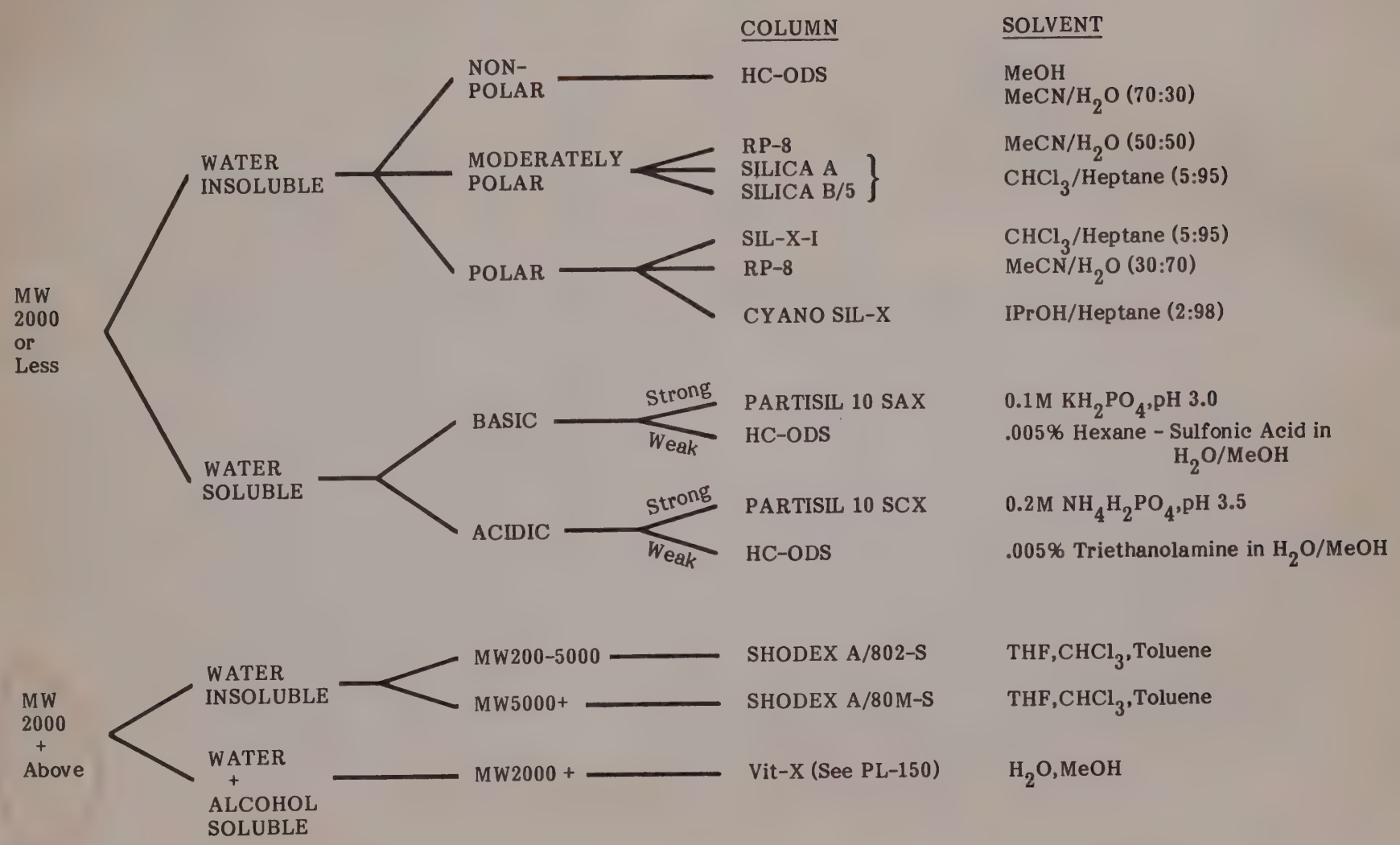
Normally, solvents are completely miscible if they are on the same half of the scale. In other words, all solvents on the upper half of the scale are completely miscible, or all on the bottom half are miscible with each other, and all solvents from the center half (1/4 to 3/4) are mutually miscible. There are several "universal" solvents such as tetrahydrofurane and acetonitrile which are miscible with almost all others except hexane and pentane. By far the most useful "rinsing, or cleanout" solvent is isopropanol, which is miscible with all others at all concentration levels.

APPENDIX II

ULTRAVIOLET ABSORPTION BANDS OF COMMON CHROMOPHORES

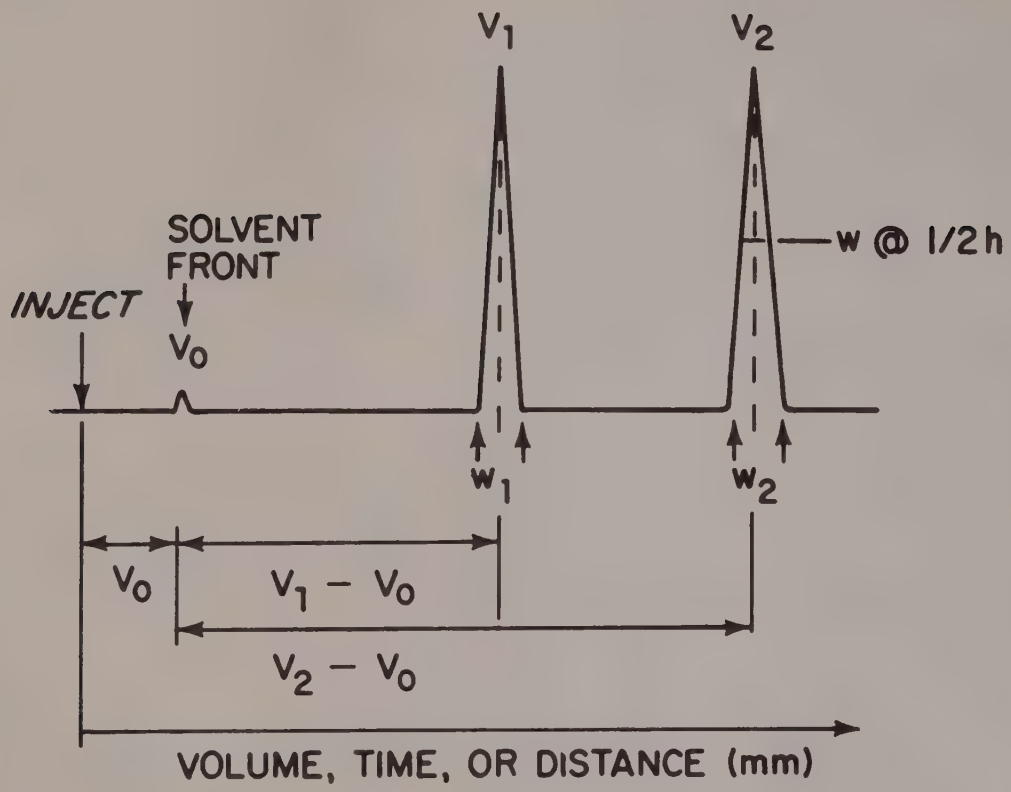
CHROMOPHORE	SYSTEM	λMAX	εMAX	λMAX	εMAX	λMAX	εMAX
Ether	-O-	185	1000				
Thioether	-S-	194	4600	215	1600		
Amine	$-NH_2$	195	2800				
Thiol	-SH	195	1400				
Disulfide	-S-S-	194	5500	255	400		
Bromide	-Br	208	300				
Iodide	-I	260	400				
Nitrile	-C≡N	160	-				
Acetylide	-C≡C-	175-180	6000				
Sulfone	$-SO_2-$	180	-				
Oxime	-NOH	190	5000				
Azido	>C=N-	190	5000				
Ethylene	-C=C-	190	8000				
Ketone	>C=O	195	1000	270-285	15-30		
Thioketone	>C=S	205	strong				
Esters	-COOR	205	50				
Aldehyde	-CHO	210	1500				
Carboxyl	-COOH	200-210	50-70				
Sulfoxide	>S→O	210	1500				
Nitro	$-NO_2$	210	strong				
Nitrite	-ONO	220-230	1000-2000	300-400	10		
Azo	-N=N-	285-400	3-25				
Nitroso	-N=O	302	100				
Nitrate	$-ONO_2$	270 (shoulder)	12				
	$-(C=C)_2-$ (acyclic)	210-230	21,000				
	$-(C=C)_3-$	260	35,000				
	$-(C=C)_4-$	300	52,000				
	$-(C=C)_5-$	330	118,000				
	$-(C=C)_2-$ (alicyclic)	230-260	3000-8000				
	C=C-C≡C	291	6,500				
	C=C-C=N	220	23,000				
	C=C-C=O	210-250	10,000-20,000			300-350	weak
	$C=C-NO_2$	229	9,500				
Benzene		184	46,700	202	6,900	255	170
Diphenyl				246	20,000		
Naphthalene		220	112,000	275	5,600	312	175
Anathracene		252	199,000	375	7,900		
Pyridine		174	80,000	195	6,000	251	1,700
Quinoline		227	37,000	270	3,600	314	2,750
Isoquinoline		218	80,000	266	4,000	317	3,500

APPENDIX III
COLUMN + SOLVENT SELECTION GUIDE



APPENDIX IV

COMMON LC CALCULATIONS



THEORETICAL PLATES(N) = $16\left(\frac{V}{w}\right)^2$ or $5.5\left(\frac{V}{w\ @\ \frac{1}{2}h}\right)^2$

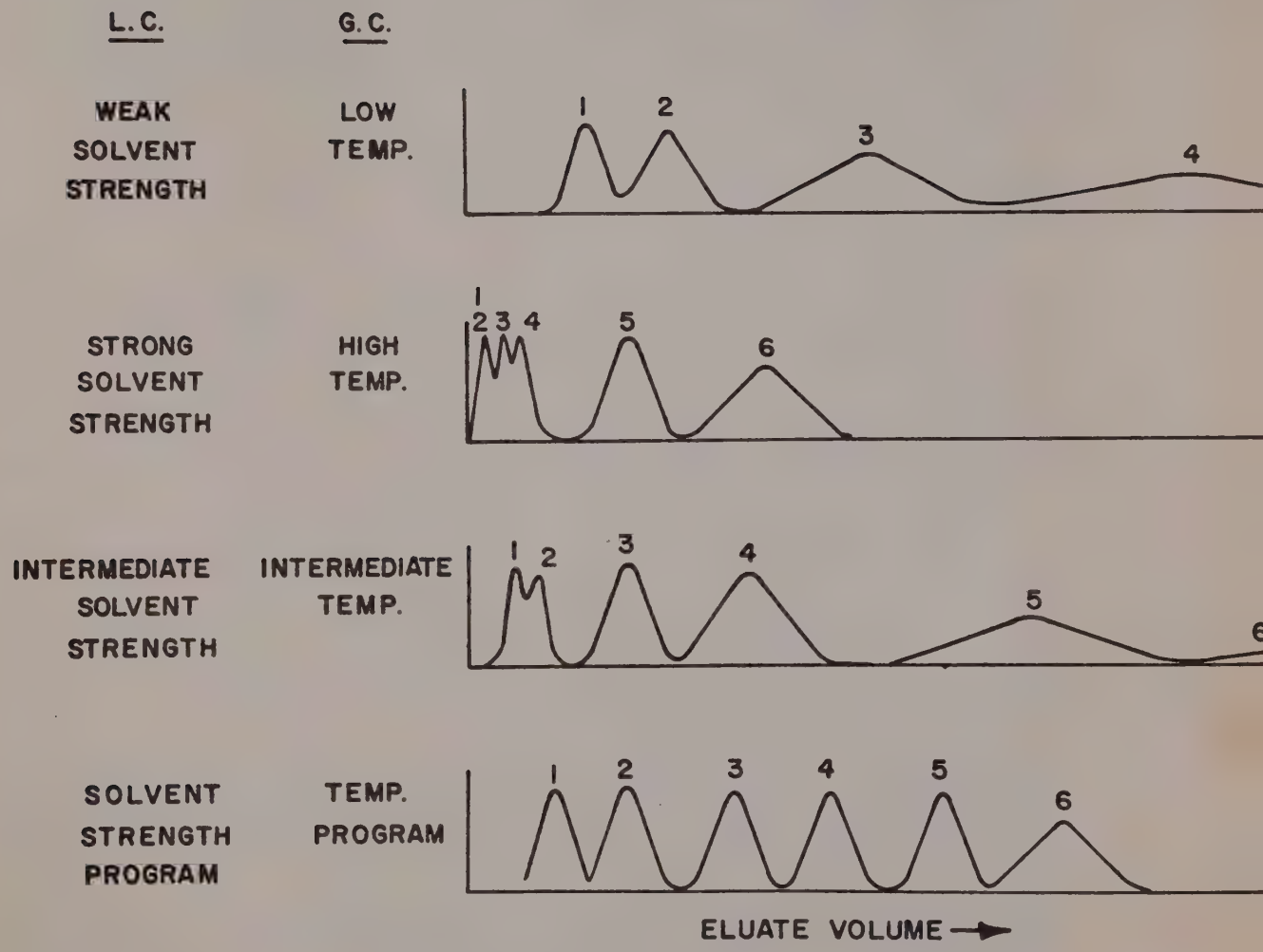
HEIGHT EQUIVALENT TO THEORETICAL PLATE(HETP) = $\frac{\text{COL. LENGTH(mm)}}{N}$

RESOLUTION (R_s) = $\frac{V_2 - V_1}{\frac{1}{2}(w_2 + w_1)}$

CAPACITY FACTOR (k') = $\frac{V_1 - V_0}{V_0}$ and $\frac{V_2 - V_0}{V_0}$

SEPARATION FACTOR(α) = $\frac{k'(V_2)}{k'(V_1)}$

THE GENERAL ELUTION PROBLEM AND ITS SOLUTION

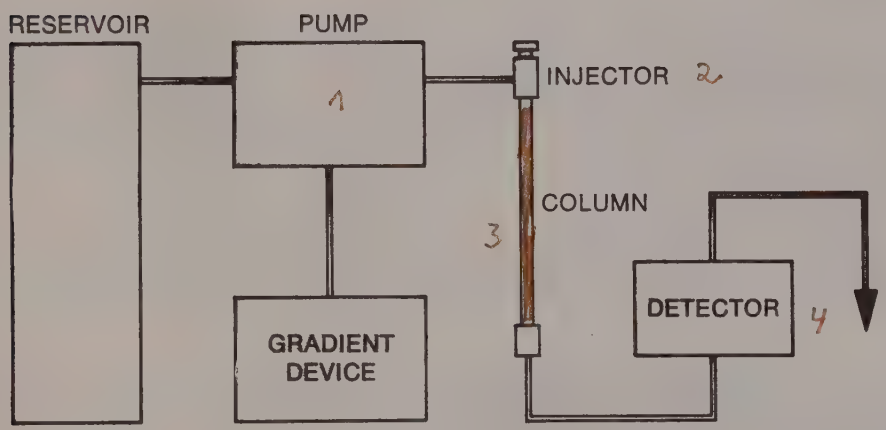


APPENDIX VI
FUNDAMENTAL CONCEPTS OF LIQUID CHROMATOGRAPHY

INTRODUCTION

Liquid chromatography, an automated, high resolution approach to column chromatography, provides the researcher with yet another separation mechanism to solve analytical problems. It enables the user to make fast, efficient separations, many previously unattainable, under mild conditions on thermally-labile and high molecular weight compounds. The potential usefulness of liquid chromatography far surpasses that of gas chromatography. While gas chromatography is applicable to approximately 15% of the known compounds, liquid chromatography is useful in analyzing the remaining 85%. In order to achieve this capability, a liquid chromatograph must be designed to allow the user to perform readily in all four modes of liquid chromatography.

THE BASIC LIQUID CHROMATOGRAPH



The basic liquid chromatograph consists of four elements. These are a pump to move the solvent carrier through the system, an injector for depositing the sample at the head of the column, the column filled with a packing material, and a detector to visualize the eluted components.

For all but the simplest of separations, a fifth capability is required, that being gradient elution or solvent programming. Solvent programming is the most powerful of all liquid chromatography parameters in increasing resolution and reducing analysis time.

The design goals of a research liquid chromatograph must be dictated by the chemistry of separation and the physics of fluids in motion. This concept must be adhered to if one is to achieve resolution of the individual components of the sample and retain this resolution until the separated species are detected and possibly collected. The pump should provide a constant pulse-free flow of solvent. The pressure increases as additional lengths of column are installed to increase resolution. Although different solvents have widely varying viscosities, when changing solvent systems the researcher does not want flow rate changes due to viscosity changes. In gas-driven syringe pumps and simple pulsating pumps, the flow rate delivered is affected by both column back pressure and solvent viscosity. Constancy and repeatability of flow rate, however, are essential in order to employ elution volume (corresponding to retention time in gas chromatography) in component identification. Smooth constant flow of solvent carrier is also necessary for best detector performance since many photometric detectors exhibit drift and noise with long and short term changes in flow rate.

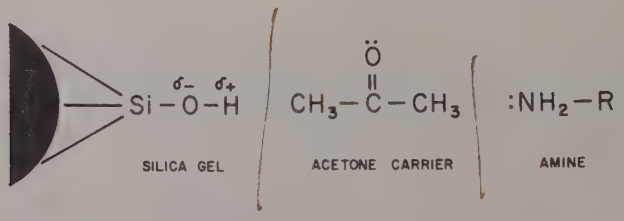
Injection devices and column end fittings must be designed with utmost care since void space in liquid chromatography has much more effect on peak broadening than in gas chromatography.

Columns require a smooth precision bore and packing which is uniform in particle size. Finally, detectors must have highly efficient flow cells which allow the separated sample components to pass through in a minimum time. Other essential characteristics are low cell volume, high sensitivity and stability, and high pressure cells, which allow back pressure to be put on the cell to prevent bubbles of dissolved gas from developing in the cell.

LC column packings are extremely small and pack tightly into the column. They offer a high amount of resistance to the flow of liquids past (and through) them. This results in relatively significant amounts of back pressure at operating flow rates.

MODES OF LIQUID CHROMATOGRAPHY

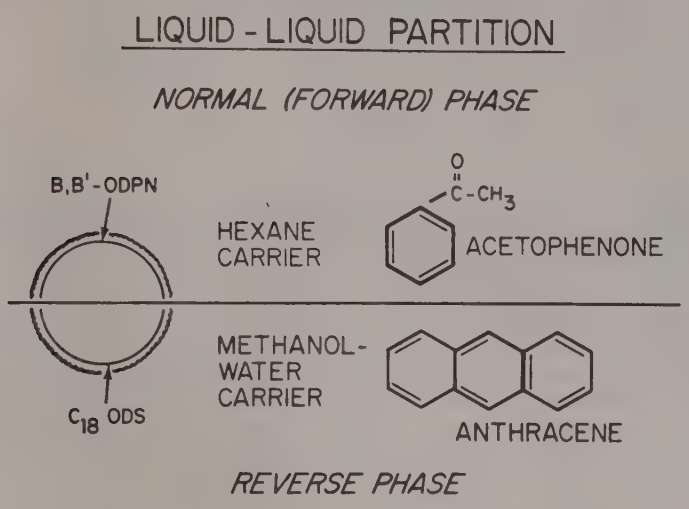
LIQUID-SOLID (ADSORPTION) CHROMATOGRAPHY



The separation mechanism in liquid-solid (adsorption chromatography) is based on the competition between the sample and the solvent molecules for the active sites on an adsorbent such as alumina or silica gel. Application is to molecules of low to high polarity up to 1000 molecular weight. Typical column lengths are 0.15 to 0.25 meters and 2 to 3 mm I.D. Back pressure at a flow rate of 1 ml./min. is about 1600 psi per meter. Mixed solvent capability is essential and gradient elution capability is useful to application of this mode since most separations are achieved by controlling the

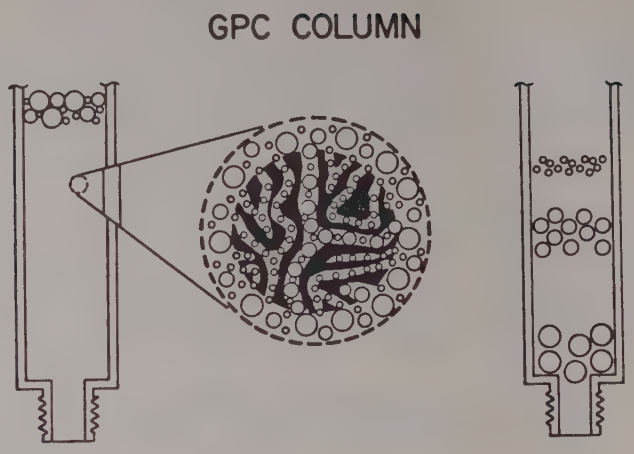
adsorptive energy of the eluent. This control is normally effected by blending, or modifying, a weak (non-polar or low adsorptive-energy) solvent with a stronger solvent, as described on the Solvent Polarity Scale (Appendix I). Since most adsorptive columns retain a coating of the more polar solvent, the separating mechanism may actually be the result of the sample partitioning between the eluting solvent and the modifying solvent which is being retained on the column. (See below.) Guidelines for solvent selection and solvent concentration adjustment are provided in Appendix I.

LIQUID-LIQUID AND BONDED PHASE PARTITION



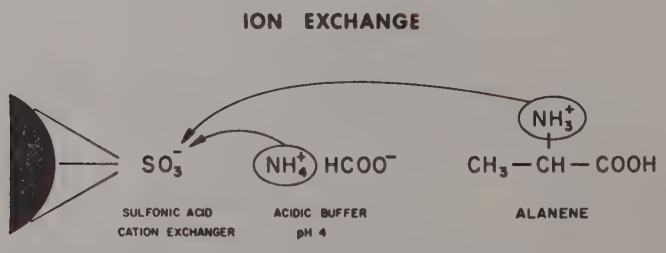
Separation in liquid-liquid partition is based on the comparative solubility of the sample molecule in the solvent and the liquid coating on a solid support. Partitioning the sample between phases delays or retains some components more than others to effect separation. Application is to moderately polar substances up to molecular weight 1000. Columns of 0.15 to 0.25 meter length (2 to 3 mm I.D.) are normally used. Typical back pressure is 1600 psi per meter at 1 ml./min. This technique requires a constant solvent flow rate independent of back pressure to permit identification on the basis of retention volume. There are two basic modes of partition chromatography: normal or forward phase, where the column surface is polar and the eluent is much less-polar, as in adsorption chromatography; and reverse phase where the column surface is non-polar and the eluent is polar (usually water modified with an alcohol or less-polar organic, such as acetonitrile). Most modern HPLC is performed on permanently-bonded stationary phases, where the stationary phase is chemically bonded to the packing. The 18-carbon octadecylsilane (ODS) bonded column is one of the most widely used types, since these columns, while ideal for non-polar samples, may also be used for moderately polar and polar samples. More recently, the shorter-chain, 8-carbon (C-8) columns have been recommended for these more-polar samples. As with the C-18 (ODS) columns, water is commonly used as the "weak" solvent and methanol or acetonitrile is added to speed elution of strongly retained compounds. Some organic soluble compounds may be so strongly retained that the solvent system may use acetonitrile as the weak solvent with THF or methylene chloride added to speed elution.

EXCLUSION CHROMATOGRAPHY



The mechanism of separation in exclusion chromatography is on the basis of physical size of sample molecules. The column is packed with a gel having pores similar in size to the molecular dimensions of the sample. Small molecules are able to permeate more pores and are therefore retained longer than large molecules. Molecular weight range of application is 200 to several million. Column lengths up to 4 meters are used. Back pressures at a flow rate of 1 ml./min. are in the range of 150 psi. This technique is applicable to polymers and other higher molecular weight samples and requires a constant, highly reproducible solvent flow rate since time of emergence of species is inversely proportional to molecular size and systems are frequently calibrated by constructing a plot of retention time vs. molecular size. Recently, columns have become available which allow high resolution of relatively low molecular weight materials and these can be used to pre-separate samples into high-and low-weight fractions.

ION EXCHANGE CHROMATOGRAPHY



Separation in ion exchange chromatography is based on the competition between the buffer solvent and the ionic or highly polar sample for sites on the ion exchange resin column packing. Application is made to a wide range of molecular weights. Peptides and amino acids are traditional examples. This technique requires 0.15 to 2 meters of 2 to 3 mm I.D. column. Typical back pressures are 800 to 2000 psi depending on speed of analysis. Ionic and/or pH gradients are essential to sweep the sample components from the column in a reasonable time.

INSTRUMENTAL REQUIREMENTS

PUMP DRIVE SYSTEMS

Pumping systems can be extremely simple, where one pump is used to provide a single solvent-or pre-mixed solvent blend, but more typically, can be more complex, where the blending of several solvents can be achieved by the pumping system. A most convenient method of achieving this utilizes two pumps, controlled by separate digital controls. This control of the pump flow rate provides a degree of flexibility and precision which is unequaled in other liquid chromatography systems, such as those which use a single pump and "blending" valves. Additional digital control circuits are provided which command these separate systems to work together to produce solvent blends and solvent programs or gradients with an infinite variety of compositions and flow rates.

SINGLE SOLVENT

The chromatographic system employing single solvent is used in exclusion chromatography and liquid-liquid partition chromatography. Essential requirements are absolute constancy of flow rate and the ability to repeat previously used flow rates in order to use the parameter of elution volume to identify species or to calculate molecular weight. Of course, as described above, a single pump can be used to deliver an eluent which is a mixture of solvents (isocratic analysis) composed to obtain the required separation. If the concentration of the mixture is improper, however, it is fairly inconvenient to change, requiring either dilution of the original, or mixing of a new blend.

MIXED SOLVENT

Mixed solvent operation is used primarily in adsorption and bonded phase chromatography. It is also useful in ion exchange work. The ability to alter the polarity of the carrier by changing the blend ratio of two different solvents saves hours of mixing and solvent changeover time. The essential parameter is constancy of flow rate despite rapid changes in carrier viscosity and the resultant changes in column back pressure.

The two separate pumps can be charged with different solvents or different ionic strength buffers, and, by means of a control designated %A in A+B, the precise fraction of blended solvent desired can be dialed into the drive control. After a preliminary run, corrections can be made on the choice of solvent blend to improve resolution and decrease analysis time. The percent of A in A+B can be varied during a run to save time in sweeping strongly retained species from the column. This gives preliminary information on the type of gradient which may be helpful in the analysis. Additionally, any ratio can be selected at any flow rate.

Mixed solvent systems are of great use to the researcher. When a non-polar solvent with adsorbent packing is used, the solvent does not readily displace polar sample molecules adsorbed on the packing, and retention times can be extremely long. With the use of a polar solvent, however, the sample molecule is readily displaced from the packing, and retention time decreases. When a mixture of two solvents is blended, the retention times can be increased until all sample components are separated.

SOLVENT PROGRAMMING

The solvent gradient is an essential capability in ion exchange and adsorption chromatography when samples cover a broad polarity range as noted in Appendix V. The solvent or buffer carrier must be changed in a predictable and highly reproducible manner in order to sweep the sample components from the column with maximum resolution and minimum time. This requires precise digital control of the pumps to achieve flexible, reproducible gradient profiles and a constant solvent flow rate despite changing solvent viscosity and the resultant change in column back pressure. Additionally, there must be no dead volume where unmixed solvent can be retained.

While more simple units allow the use of strictly linear program changes, where the rate of change, or total time, is the only variable, more complex instruments include curved gradient capability.

Solvent gradients from convex through linear to concave can be selected and adjusted through a wide range of curvatures. Convex, linear, and concave gradients and the degree of curvature are selected from the instrument control console. Since the gradients are generated by digital techniques, the long term precision of the system is assured.

To form a solvent gradient, the two pumps are charged with solvents of different polarity or ionic strength which represent the limits which should be necessary to remove all the sample species from the column. One pump starts pumping at the preselected flow rate while the second is stopped. As the first pump is reduced in speed, the second picks up speed so that the two separate flow rates always add (or complement) to produce a constant flow rate. In this manner, the output solvent composition is smoothly and reproducibly varied from the first solvent to the second by any linear or non-linear function. As in mixed solvent operation, the flow rate is determined by a separate control so that the program can be generated at any desired flow rate. In some applications, solvent concentration is held constant and the flow rate is programmed, beginning with very slow flow to elute weakly-retained species, and gradually increasing the flow rate to elute more strongly-retained components.

DUAL CHROMATOGRAPH OPERATION

Dual pump systems can be used completely independently so that, by the addition of a second column injector assembly and a second detector, two separate problems or two aspects of the same problem can be pursued at the same time. This mode of operation requires two totally separate pumps including controls. Perkin-Elmer Series 2 and 3 LC Pump Modules permits this if a second column, injector and detector are fitted.

COLUMNS AND INJECTORS

Radial diffusion in gas chromatography systems tends to reduce transcolumn effects. That is, when moving from one diameter conduit to another, radial diffusion helps sweep the material out of the corners. Radial diffusion in a liquid stream is only about 1/10,000 of that in a gaseous stream, however. For this reason, conduits must be kept as small and as constant as possible, and in zones where the conduit diameter must change, special consideration must be given to the zone of transition from one diameter to the other.

The method of injection is naturally critical in liquid chromatography. Several methods are in common use. The first is a high pressure loop injector available with variable volume loops and fixed internal loop versions available in the range 2 to 10 microliters. These injectors are designed with a uniform bore throughout the injector body, seat, and loop to eliminate back mixing of the sample. Other valve injectors allow the applications of various amounts of sample without loop change. A third method of injection is an on-column septum injector designed to permit repetitive syringe injections at pressures up to 2000 psi.

DETECTORS

The design of liquid chromatography detectors requires more than just high sensitivity. Low dead volume is necessary to prevent unwanted diffusion or band spreading. The basic cell design must be of an efficient nature to allow rapid flushing with minimal volumes of solvent. Dynamic range must be wide to provide the high sensitivity required in analytical work while retaining the ability to be attenuated on-scale with large preparative samples. Finally, a high degree of stability is required since this determines the accuracy of quantitation.

The most common LC detectors are those which detect changes in ultraviolet (UV) absorption. Typically these include an energy source beaming light through a long, narrow flowcell through which column effluent is pumped. Non-absorbing solvents are used so the normal baseline represents maximum energy resulting from all of the light reaching the photodetector which is on the opposite side of the cell from the light source. Some light is lost when compounds which have the various types of molecular structure, or contain functional groups, which absorb UV light at the wavelength in use, pass through the cell. The resultant change in energy is converted to an increase in millivolt output which is then displayed on a strip chart recorder and/or is integrated on data acquisition devices.

The greater the concentration of the absorbing compound in the cell, the more light is absorbed, thus the larger the signal. In addition, since the degree of absorption is dependent upon the specific structure or functional groups present (molar absorptivity), signal intensity is also a function of the type of compound being detected. This can vary by a factor of thousands, as noted in Appendix II. Thus, strict standardization procedures must be used to determine the actual response of each component.

UV detectors range from simple single wavelength units which obtain energy, primarily at 254 nm (where about 60% of all organics have some absorption from low pressure mercury lamps; to more widely applicable monochromator equipped units utilizing broad-spectrum deuterium and tungsten light sources. With these latter units, any wavelength between 190 and 800 nm may be selected, to take advantage of the optimum UV absorption characteristics of specific sample species. (See Appendix III.) This allows detection of over 90% of all organic compounds.

The Perkin-Elmer UV detectors were designed with low volume, high efficiency cells coupled to highly stable electronics possessing wide dynamic range to provide the ultimate in liquid stream detection. Additional benefits include high pressure capability which allows back pressure to be placed on the cells, often eliminating the need for solvent degassing.

REFRACTIVE INDEX DETECTORS

Another detection mode is Refractive Index, where a dual-cell detector allows continuous comparison of a reference stream, containing only eluting solvent, with the sample stream. As sample components elute, they refract light at different angles, resulting in changes of energy at the photodetector, and in changes in output signal as described previously. While the UV detector ideally measures changes of light energy in a single cell, and required no equilibration or flushing, the RI detector is strictly a comparative technique, where the output is always the difference between the two cells. It is therefore difficult, or practically impossible, to change solvents during runs, and requires that the reference side be purged and re-equilibrated each time the slightest solvent concentration change is performed.

FLUORESCENCE DETECTORS

About 20% of the known organic compounds fluoresce (emit high-wavelength energy when excited by low-wavelength energy). When this property exists, fluorescence detectors which allow selection of optimum excitation and emission wavelengths (as described in Perkin-Elmer's publication "Introduction to Fluorescence" (TN-60)), provide high degrees of selective and sensitive detection.

DETECTOR COUPLING

If zero dead volume unions and extremely narrow-bore tubing are used, (0.015-inch or less) detectors may be coupled in series. Ideally, since its cell can withstand more back pressure than RI or fluorescence detectors, the UV detector should be first, RI second, and fluorescence third.

APPLICATION

While the RI detector is most general (everything has a refractive index), maximum sensitivity is limited to about one microgram of material. Fluorescence detection is most sensitive, with strong-fluorescing compounds detectable to the sub-picogram level, but most limited in its application. The best combination of applicability and sensitivity is found in the variable-wavelength UV detector where typical detection capability is in the nanogram region for most strong-absorbing compounds.

TEMPERATURE CONTROL

Increasing the effective operating temperautre in LC has two important effects. First, mass transfer between the phases is increased, resulting in sharper and more symmetrical peaks (better separations). Second, the unique retention time for each sample component will be decreased; i.e., it will elute from the column more quickly. However, the same temperature change will not affect each sample component to the same degree.

Temperature control is essential for achieving reliable and reproducible separations by liquid chromatography. It is an important variable that can be used routinely to:

- Provide better quality separations through improving both column efficiency and peak symmetry.

- Optimize separation time. Elevated temperatures reduce solvent viscosity and therefore generally permit the use of higher flow rates (shorter run times) within back pressure limitations.

- Increase the reliability of qualitative analysis in LC based on peak retention times.

Common operating temperatures in LC are in the 60 to 79 degree centigrade region. This is normally sufficient to achieve the desired enhancement of solubility, reduction of viscosity, and elimination of ambient-change induced variances.

SAMPLE PREPARATIONS AND QUANTITATION

Many samples may be simply dissolved in a suitable solvent and injected directly into the instrument. If samples contain particulate material, or components which may bond to, or alter, the column (such as the proteins in plasma and urine samples) they should be either filtered through a micro-porous filter such as the Perkin-Elmer 0089-0835 or 0089-0837 (see PL-409) and/or extracted or filtered through ion-exchange media. Sample and solvent workup procedures are thoroughly discussed in Perkin-Elmer's publications TN-69 and LC-100. LC-100 also includes information on the use of LC in quantitative analysis.

PREPARATIVE SCALE OPERATION

After successful analytical separation of a sample is achieved, it is often required that the system be scaled up to allow sufficient material to be processed so that subsequent analysis for identification or experimentation can be performed. This mode of operation requires large column capacity, increased solvent capacity, and sample collection capability. Solvent flow rate should be constant, and sample collection actuation must operate on a precise basis. This allows immediate and non-ambiguous identification and location of the sample components in the corresponding vials of the fraction collector, referenced to the recorder trace of the detector output.

SELECTION OF SOLVENT AND DETECTION WAVELENGTH.

UV absorption spectra of compounds can be used to determine optimum wavelength settings. Compound A can be seen to have high absorption at 260 nm; Compound B, at 330. Both have good absorbance at 255 thus this may be the best "compromise" wavelength if both are to be detected in the same run. Note that both have very high absorption in the far UV; however if the solvent system contains chloroform, it can be seen that the chloroform would saturate the system below 230, thus the far UV cannot be used in this case. If maximum detection of each compound is desired, it is sometimes useful to change wavelengths during the run. Note too that at 255 nm the chloroform still absorbs some UV light while the hexane does not. This means that a baseline shift will be noted if a solvent ratio change is performed.

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